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file  
in  
SF4941  
Thanks  
Dennis*

MEMORANDUM

DATE: 22-NOV-1999

SUBJECT: PP# 8F04941. New Chemical- Prohexadione-Calcium, in/on Peanuts, Pome Fruits, and Cattle Meat Byproducts (Kidney). **Evaluation of Residue Data and Analytical Methods.** MRID#s 444578-03 thru -06. Barcodes D252547 & D253640. Chemical No 112600. Cases 289440 & 064306. Submissions S557351 & S555057.

FROM: George F. Kramer, Ph.D., Chemist  
RAB1/HED (7509C)

THRU: Melba Morrow, D.V.M., Branch Senior Scientist  
RAB1/HED (7509C)

TO: Cynthia Giles-Parker  
Registration Division (7505C)

K-I Chemical U.S.A. has submitted a petition for the establishment of permanent tolerances for residues of a new plant growth regulator, prohexadione-calcium (calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate), in/on peanuts, pome fruits, and cattle meat byproducts (kidney). Concurrently, the petitioner is requesting Section 3 registration for end-use products containing prohexadione-calcium as the active ingredient; a 27.5% dry flowable (DF) formulation (Product Name = Apogee™, EPA File Symbol No. 63588-RR); and a 75% DF formulation (Product Name = Baseline™; EPA File Symbol No. 63588-O). Apogee™ is intended for use on apple and pear trees and Baseline™ is intended for use on peanuts. Section F of the petition proposes the establishment of tolerances for residues of prohexadione-calcium *per se* [cyclohexanecarboxylic acid 3,5-dioxo-4-(1-oxopropyl)-ion(1-) calcium, calcium salt] in/on the following commodities:

|   |         |
|---|---------|
| Peanut Nutmeat . . . . .                  | 1.0 ppm |
| Peanut Hay . . . . .                      | 0.6 ppm |
| Pome fruit . . . . .                      | 3.0 ppm |
| Cattle, Meat Byproduct (Kidney) . . . . . | 0.1 ppm |

The attached contractor's document has been reviewed and revised to reflect HED policy.

#### Executive Summary of Chemistry Deficiencies

- A revised Baseline™ label, which includes a 30-day plantback restriction for all rotational crops.
- Determination of residue of concern by HED Metabolism Assessment Review Committee (MARC).
- Agency validation of analytical enforcement method for plants.
- Need storage stability data on apples and the processed commodities of peanuts.
- Revised Section F.

#### RECOMMENDATIONS

HED concludes there are no residue chemistry data requirements that would preclude the establishment of permanent tolerances for residues of prohexadione-calcium in/on pome fruit, peanut and animal RACs provided that: 1) Sections B and F are revised as specified in Conclusions 2, 14, 15a and 18a; 2) prohexadione-calcium *per se* is determined to be the only residue of concern by the HED MARC; and 3) Agency validation of analytical enforcement method for plants is successful. Registration of Apogee™ and Baseline™ should be made conditional upon the submission of additional storage stability data as specified in Conclusions 12c and 12d. A human-health risk assessment will be prepared as a separate document.

Attachment 1- contractor review

cc: PP#8F04941, Kramer, R.F.  
RDI: Chem SAC (11/17/99), M. Morrow (//99), Team (10/28/99)  
G.F. Kramer:806T:CM#2:(703)305-5079:7509C:RAB1

PROHEXADIONE-CALCIUM  
PC Code 112600  
(DP Barcodes D252547)

PP#8F04941: Evaluation Of Residue Chemistry Data  
To Support Permanent Tolerances For Prohexadione-  
Calcium  
On Peanuts, Pome Fruits And Cattle, Meat  
Byproduct (Kidney)

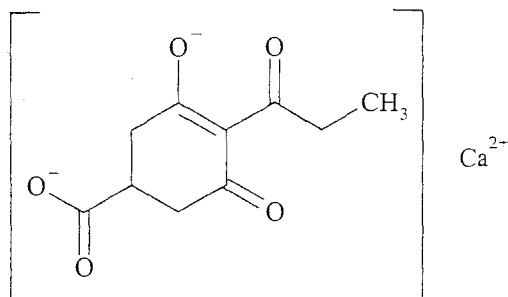
June 11, 1999

Contract No. 68-D4-0010

Submitted to:  
U.S. Environmental Protection Agency  
Arlington, VA

Submitted by:  
Dynamac Corporation  
The Dynamac Building  
2275 Research Boulevard  
Rockville, MD 20850-3268

## PROHEXADIONE-CALCIUM



PC Code 112600

PP#8F04941: EVALUATION OF RESIDUE CHEMISTRY DATA TO SUPPORT

PERMANENT TOLERANCES FOR USE OF PROHEXADIONE-CALCIUM

ON PEANUTS AND POME FRUITS

DP Barcodes D252547 & D253640

### INTRODUCTION

K-I Chemical U.S.A. has submitted a petition for the establishment of permanent tolerances for residues of a new plant growth regulator, prohexadione-calcium (calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate), in/on peanuts, pome fruits, and cattle meat byproducts (kidney). The residue chemistry data submitted in support of the petition were generated by BASF Corporation. Concurrently, the petitioner is requesting Section 3 registration for end-use products containing prohexadione-calcium as the active ingredient; a 27.5% dry flowable (DF) formulation (Product Name = Apogee™, EPA File Symbol No. 63588-RR); and a 75% DF formulation (Product Name = Baseline™, EPA File Symbol No. 63588-0). Apogee™ is intended for use on apple and pear trees and Baseline™ is intended for use on peanuts. Prohexadione-calcium is effective in controlling vegetative growth in pome fruits and peanuts by inhibiting gibberellin biosynthesis. In peanuts, prohexadione-calcium is effective in canopy control, which facilitates row location for mechanical harvesting; in pome fruits, prohexadione-calcium improves fruit quality by allowing increased sunlight penetration into the canopy.

Section F of the petition proposes the establishment of tolerances for residues of prohexadione-calcium *per se* [cyclohexanecarboxylic acid 3,5-dioxo-4-(1-oxopropyl)-ion(1-)] calcium, calcium salt] in/on the following commodities:

|   |         |
|---|---------|
| Peanut Nutmeat . . . . .                  | 1.0 ppm |
| Peanut Hay . . . . .                      | 0.6 ppm |
| Pome fruit . . . . .                      | 3.0 ppm |
| Cattle, Meat Byproduct (Kidney) . . . . . | 0.1 ppm |

Representatives from the U.S. EPA and BASF Corporation held a meeting on 6/17/97 to discuss residue chemistry and toxicology data requirements necessary for the registration of prohexadione-calcium on peanuts. The petitioner presented data from the peanut field trials, freezer storage stability, and method accountability studies. The metabolism of prohexadione-calcium on peanuts was discussed including an interpretation of the importance of metabolites characterized and identified in the study. The meeting resulted in the issuance of a Memorandum of Understanding (MOU) dated 10/6/97. A copy of the MOU was included as an appendix by the petitioner in the administrative materials of this petition.

Associated with this petition are 21 volumes of residue chemistry submissions which are evaluated in this document.

### CONCLUSIONS

#### OPPTS 830 Series GLNs: Product Properties

1. The review of product chemistry data associated with this petition is under the purview of RD.

#### OPPTS GLN 860.1200: Proposed Uses

2. The proposed uses of prohexadione-calcium on apples and pear (the representative commodities of pome fruits) were adequately described on the Apogee™ label. **However, a revised Baseline™ label, which includes a 30-day plantback restriction for all rotational crops (except peanuts), should be submitted.**

#### OPPTS GLN 860.1300: Nature of the Residue in Plants

##### Apple:

- 3a. The apple metabolism study is acceptable. Total radioactive residues were 0.305 ppm in/on mature apples harvested 45 days following the last of two sequential applications of [<sup>14</sup>C]prohexadione-calcium, labeled at the C-3 and C-5 positions of the cyclohexenone ring, at a total application rate of 1.76 lb ai/A (~1x the maximum proposed seasonal rate). Applications were made directly to apples as a run-off spray.
- 3b. Over 94% of the total radioactive residues (TRR) in/on mature apples were characterized and identified. The characterization/identification of the organosoluble fractions indicated that the radioactive residues consisted of several minor components, none greater than 12% of the TRR. Prohexadione-calcium was rapidly metabolized to prohexadione (free acid), which was identified at 1.83% TRR (0.0056 ppm).

Metabolites BX 112-I5 and BX 112-M10 were the major identified metabolites, accounting for 11.78% TRR (0.0359 ppm) and 9.21% TRR (0.0281 ppm), respectively. The following metabolites were also identified: despropionyl prohexadione (5.55% TRR, 0.0169 ppm), 27F2-B (7.68% TRR, 0.0234 ppm), 25F1-A (5.33% TRR, 0.0163 ppm), 27F2-A (2.60% TRR, 0.0079 ppm), tricarballic acid (1.24% TRR, 0.0038 ppm), and citric acid (2.44% TRR, 0.0074 ppm). Organosoluble unknowns (20 components) accounted for 36.32% TRR and aqueous-soluble unknowns (6 components) accounted for 5.92% TRR. The petitioner demonstrated that the remainder of the characterized radioactivity was distributed between base hydrolysate unknowns <3000 MW (5 components) at 1.32% TRR, base hydrolysate unknowns >3000 MW at 1.29% TRR, and glucose osazones at 2.20% TRR. The chemical structures of metabolites identified in plant and animal metabolism studies are depicted in Figure 1 (Attachment II).

#### Peanut:

- 4a. The peanut metabolism study is acceptable. Total radioactive residues were 4.15 ppm in peanut nutmeats, 2.50 ppm in hulls, and 36.5 ppm in hay collected 22 days following an over-the-top postemergence spray of [<sup>14</sup>C]prohexadione-calcium labeled at the C-3 and C-5 positions of the cyclohexenone ring at ~1.0 lb ai/A (~2.7x the maximum proposed seasonal rate of 0.375 lb ai/A).
- 4b. Over 70% (nutmeats), 52% (hulls), and 61% (hay) of the TRR were characterized and identified. Prohexadione-calcium was rapidly metabolized to prohexadione (free acid). Prohexadione was the major residue identified in nutmeats (38.3% TRR, 1.58 ppm), hulls (9.66% TRR, 0.24 ppm), and hay (35.08% TRR, 12.80 ppm). The dioxopropyl prohexadione metabolite was identified in hulls (7.48% TRR, 0.19 ppm), nutmeats (2.08% TRR, 0.09 ppm) and hay (5.79% TRR, 2.12 ppm). The following additional metabolites were identified: despropionyl prohexadione in hulls (2.54% TRR, 0.064 ppm) and hay (1.69% TRR, 0.615 ppm); and TCA in nutmeats (3.06% TRR, 0.13 ppm), hulls (15.92% TRR, 0.40 ppm), and hay (12.66% TRR, 4.62 ppm).
- 4c. Two unknowns, HA5 and HA6, characterized as oxidation products similar to dioxopropyl prohexadione accounted, respectively, for 5.63% and 6.01% TRR in nutmeats, 2.45% and 1.84% TRR in hulls, and 1.27% and 1.15% TRR in hay. In addition, residues characterized as lipids accounted for 3.01% TRR in nutmeats; residues characterized as lignin accounted for 2.61% and 3.70% TRR, respectively, in hulls and hay. The nonextractable residues in nutmeats, hulls, and hay were characterized as base-labile conjugates of prohexadione and its metabolites, sugars (osazones), carbohydrates, proteins, and polar metabolites <3000 MW.

#### Plant metabolism conclusions:

5. The qualitative nature of the residue of prohexadione-calcium in plants is adequately understood for the purpose of this petition. The metabolism of prohexadione-calcium in apples and peanuts is similar. Prohexadione-calcium is rapidly metabolized to prohexadione and parent-like oxidative intermediates and ultimately to tricarballic acid (TCA), citric acid, and other natural products from the plant carbon pool. RAB1 will defer to the HED Metabolism Assessment Review Committee (MARC) on the toxicological significance of the prohexadione-calcium metabolites. A decision concerning which residues to regulate will then follow. A tolerance based on the parent only may not be appropriate; in such an instance a revised Section F and additional field studies, analytical methodology, and storage stability data may be needed.

#### OPPTS GLN 860.1300: Nature of the Residue in Animals

##### Ruminants:

- 6a. The goat metabolism study is acceptable. Following oral administration of [<sup>14</sup>C]prohexadione-calcium to lactating goats for four consecutive days at 105 ppm, the TRR were <0.0604-0.0884 ppm in milk, 3.09-3.16 ppm in kidney, 0.427-0.432 ppm in liver, 0.061-0.069 ppm in muscle, and 0.048-0.054 ppm in fat. The feeding level of 105 ppm is equivalent to ~52x the anticipated maximum dietary burden of 2.0 for dairy cattle and ~31x the anticipated maximum dietary burden of 3.4 ppm for beef cattle.
- 6b. Approximately 71-103% of the TRR were characterized/identified in milk and tissues. Prohexadione-calcium was converted to prohexadione as the free acid. Prohexadione was the major residue identified in milk (19.0% TRR, 0.016 ppm), kidney (40.9% TRR, 1.267 ppm), liver (30.6% TRR, 0.131 ppm), muscle (73.5% TRR, 0.051 ppm), and fat (89.3% TRR, 0.043 ppm). Metabolites related to, or a precursor of, despropionyl prohexadione were identified in kidney (31.7% TRR, 0.980 ppm) and liver (10.5% TRR, 0.045 ppm). Polar and low molecular weight metabolites were characterized as carboxylic acids in liver (20.2% TRR, 0.086 ppm). Milk contained radioactivity incorporated into sugars (22.6% TRR, 0.019 ppm) and lipids (15.2% TRR, 0.012 ppm), suggesting metabolism of prohexadione to the carbon pool.

##### Poultry:

- 7a. The hen metabolism study is acceptable. Following oral administration of [<sup>14</sup>C]prohexadione-calcium to laying hens for five consecutive days at 8.43 ppm (~34x the maximum theoretical dietary burden for poultry), the TRR in eggs and tissues each were <0.01 ppm. In the same study, following oral administration of [<sup>14</sup>C]prohexadione-calcium at 33.4 ppm

(~134x), the TRR were <0.008-0.019 ppm in egg yolks, <0.005-0.015 ppm in egg whites, 0.021 ppm in gizzard, 0.475-0.471 ppm in kidneys, 0.0289-0.030 ppm in liver, <0.007 ppm in breast muscle, 0.010-0.011 ppm in thigh muscle, <0.01 ppm in fat, and 0.022 ppm in skin with fat.

- 7b. The levels of extractable residues in eggs and tissues of hens dosed at 33.4 ppm were mostly  $\leq 0.01$  ppm. Prohexadione was the major residue identified in egg yolks (12.5% TRR, 0.003 ppm), egg whites (27.3% TRR, 0.004 ppm), liver (14.9% TRR, 0.005 ppm); and kidney (13.2% TRR, 0.062 ppm). Tricarballic acid was identified in kidney (15.5% TRR, 0.073 ppm). An unknown component, designated as Metabolite 1, was resolved in egg whites (71.5% TRR, 0.010 ppm), egg yolks (2.3% TRR, 0.001 ppm), and liver (18.2% TRR, 0.006 ppm). Attempts to identify Metabolite 1 using a combination of techniques including acid, base, and/or enzyme hydrolysis, methylation, HPLC, GC/MS and LC/MS were unsuccessful; further attempts were not performed because of insufficient material.

#### Animal metabolism conclusions:

8. The qualitative nature of the residue in animals is adequately understood. In ruminants, prohexadione is metabolized to despropionyl prohexadione metabolites or precursors and then to despropionyl prohexadione. The subsequent metabolism of despropionyl prohexadione then yields low molecular weight carboxylic acid and finally incorporation into naturally occurring products such as sugars, lipids and proteins. In poultry, prohexadione is metabolized to tricarballic acid and then to natural products such as proteins. RAB1 will defer to the HED MARC on the toxicological significance of the prohexadione-calcium metabolites. A decision concerning which residues to regulate will then follow. A tolerance based on the parent only may not be appropriate; in such an instance a revised Section F and additional field studies, analytical methodology, and storage stability data may be needed.

#### OPPTS GLN 860.1340: Residue Analytical Method - Plant Commodities

- 9a. To measure residues of prohexadione-calcium in plants, the petitioner has developed residue analytical methods using GC and a mass selective detector (GC/MSD). These GC/MSD methods are designated Methods D9601 and D9608. Method D9601 was first developed to measure residues of prohexadione-calcium in/on peanut nutmeat and hay. Method D9608 is identical to Method D9601, and was later developed to include instructions for the analysis of residues in/on pome fruits and animal commodities. The methods are proposed for tolerance enforcement, and were used as the data-collection methods in the analyses of samples obtained from the field, processing, and storage stability studies. Both methods were designed to



measure residues of prohexadione-calcium as the prohexadione methyl ester (designated by company codes BW 125-M7 or BW9054-M7). Residues of prohexadione-calcium are calculated by multiplying a molecular weight correction factor (MWCF) of 1.107 by the quantitated residues of prohexadione methyl ester. The MWCF of 1.107 was derived by multiplying 0.938 (the MWCF from prohexadione methyl ester to prohexadione) and 1.18 (the MWCF from prohexadione to prohexadione-calcium). The reported LOQ for prohexadione-calcium is 0.05 ppm for all apple, pear, and peanut commodities.

9b. To assess the significance of the oxidative metabolite BX112-I5 (which is referred to as BW125-31F in pome fruit field trials) and to assist HED in determining whether or not the compound should be included as part of the tolerance expression, the petitioner developed an HPLC/UV method, designated as Method D9810. This metabolite was identified in mature fruits (11.78% TRR, 0.0359 ppm) collected from the apple metabolism study. The reported LOQ for BX 112-I5 was 0.05 ppm for apple and pear commodities.

9c. The concurrent method recovery data indicate that GC/MSD Methods D9601/D9608 and HPLC/UV Method D9810 are adequate for data collection. In a specificity study, no interferences were reported when Method D9601 was tested using other pesticide compounds registered for use on apples and peanuts. Method D9601 was successfully radiovalidated using samples from a 1993 peanut metabolism study. Method D9601 was also subjected to a successful independent laboratory validation. The method and ILV have been sent to the Agency Analytical Chemistry Laboratories (ACL) in Fort Meade for a Petition Method Validation (PMV) (Memo, G. Kramer 8/3/99; D257929). RAB1 will withhold a final conclusion on the adequacy of this method as an analytical enforcement method pending receipt of the PMV report.

OPPTS GLN 860.1340: Residue Analytical Methods - Animal Commodities

10a. The petitioner proposes Method D9608 as an animal enforcement method. The method can determine residues of prohexadione-calcium and despropionyl prohexadione in animals. Quantitation of prohexadione-calcium is by GC/MSD, and quantitation of the despropionyl metabolite is by HPLC/UV. The reported LOQ for prohexadione-calcium was 0.05 ppm for liver, kidney, fat, and muscle and 0.01 ppm for milk. The reported LOQ for despropionyl prohexadione was 0.05 ppm for liver and kidney.

10b. Method D9608 was used as the data-collection methods in the analyses of samples obtained from ruminant feeding study. The

concurrent method recovery data indicate that Method D9608 is adequate for data collection. In a specificity study, no interferences were reported when Method D9608 was tested using other pesticide compounds with established tolerances on meat (kidney and liver). Method D9608 was successfully radiovalidated using samples from a goat metabolism study. The results of the independent laboratory validation was also successful. The method and ILV have been sent to ACL for PMV (Memo, G. Kramer 8/3/99; D257929). HED will withhold a final conclusion on the adequacy of this method as an analytical enforcement method pending receipt of the PMV report.

OPPTS GLN 860.1360: Multiresidue Method

11. The petitioner submitted data (MRID 44457802) concerning the recovery of residues of prohexadione and its despropionyl metabolite using FDA multiresidue method protocols (PAM Vol. I). These data will be forwarded to FDA for complete evaluation. The petitioner reported that residues of prohexadione and the despropionyl metabolite are not recovered using FDA multiresidue method protocols.

OPPTS GLN 860.1380: Storage Stability Data

Plant commodities:

- 12a. The maximum storage intervals (from harvest to analysis for residues of prohexadione-calcium) of samples from the field and processing studies were as follows: apples and pears (3 months), peanut hay and nutmeat (7 months), apple juice and wet pomace (1 month), and peanut meal and refined oil (7 months; 6.5 months as the RAC homogenate and <1 month as the processed commodity). The maximum storage interval (from harvest to residue analysis of metabolite BX 112-15) of samples of apples from the processing study was 26 months.
- 12b. Storage stability data are available for peanut nutmeat and hay. These data indicate that weathered residues of prohexadione-calcium are relatively stable in/on peanut hay when stored under frozen conditions for up to 12 months. In peanut nutmeat, residues of prohexadione-calcium initially declined (~50%) between 1- and 3-month intervals but stabilized (30-43% residue decline) at the 6-, 9-, and 12-month intervals. Provided that residues are adjusted to account for residue decline in peanut nutmeat during storage, these data support the storage intervals of samples collected from the peanut field trials.
- 12c. According to the petitioner, no storage stability data were submitted for pome fruits because initial analyses of field samples were completed within one month. HED, however, notes that some residues reported in the pome field trials include

values for samples which were re-analyzed after 3 months of storage. Given the observed instability of prohexadione-calcium residues in/on peanut nutmeat, the petitioner is required to provide confirmatory storage stability data on pome fruits reflecting an interval of three months. The petitioner has indicated that a freezer storage stability study for prohexadione-calcium and the metabolite BX-112-15 in apples has been initiated and is scheduled to be completed by November 2000.

- 12d. No storage stability data are required for the processed commodities of apples because analyses of samples were completed within one month of sampling. However, storage stability data on the processed commodities of peanuts, reflecting an interval of 7 months, are required.

Animal commodities:

13. No storage stability data are required for milk and ruminant tissues because analyses of samples, collected from the ruminant feeding study, were completed approximately within one month of sampling.

OPPTS GLN 860.1500: Crop Field Trials

Peanuts:

14. Residues of prohexadione-calcium were ~~<0.05-0.896~~ ppm in/on peanut nutmeat and ~~<0.05-0.539~~ ppm in/on peanut hay harvested 25 days (the proposed PHI) following the last of three broadcast foliar applications of the 75% DF formulation at 0.125 lb ai/A/application (0.375 lb ai/A/season; 1x). The 1997 residue decline data indicate that residues of prohexadione-calcium dissipate with longer preharvest intervals. Residues were highest in the 15-day PHI and declined to the lowest levels at the 25-, 35-, and/or 45-day PHIs. HED concludes that the petitioner has provided adequate residue data. The results of the 1997 peanut field trials, wherein samples were analyzed within 30 days of harvest to minimize residue degradation, support the proposed tolerances of 1.0 ppm in/on peanut nutmeat and 0.6 ppm in/on peanut hay. However, the correct commodity definitions are "Peanut" and "Peanut, hay." The Agency has also determined that "calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate" is the preferred chemical name for prohexadione-calcium (Memo 5/18/99, H. Podall; D253852). **A revised Section F is required.**

Pome fruits:

- 15a. Residues of prohexadione-calcium were <0.05-2.631 ppm in/on apples harvested 45-46 days (the proposed PHI) following the last of two broadcast foliar applications of the 27.5% DF formulation at 0.85 lb ai/A/application (1.7 lb ai/A/season;

1x). Residues of prohexadione-calcium were 0.23-0.99 ppm in/on pears harvested 44-45 days following a single broadcast foliar application of the 27.5% DF formulation at 1.7 lb ai/A (1x). The petitioner has provided adequate residue data reflecting the maximum proposed use pattern for prohexadione-calcium on pome fruits. **Pending submission of confirmatory storage stability data**, the residue data submitted for apples and pears support the establishment of the proposed crop group tolerance for residues of prohexadione-calcium in/on pome fruits at 3.0 ppm. However, the correct commodity definition is "Fruit, pome, group." **A revised Section F is required.**

- 15b. Residues of the oxidative metabolite BX 112-I5, which was detected in the apple metabolism study, were less than the LOQ (<0.05 ppm) in all treated samples of apple and pear harvested 45 days following treatment at 1x the maximum proposed seasonal application rate. The petitioner proposes that these data represent field conditions, and therefore, the metabolite BX 112-I5 is not a residue of concern.
- 15c. Additional data were provided demonstrating residues of prohexadione-calcium in/on apples treated at 2x the maximum proposed seasonal application rate. Residues of prohexadione-calcium were <0.05-6.443 ppm in/on apples harvested 45-46 days following the last of two broadcast foliar applications of the 27.5% DF formulation at 1.7 lb ai/A/application (3.4 lb ai/A/season; 2x). These data demonstrate that residue levels are proportional to the application rate.
- 15d. Residue decline data indicate that generally, residues of prohexadione-calcium decline over time. Residues were highest at the 10-day PHI and declined to the lowest levels at the 45- and 55- (apples) or 65-day (pears) PHIs.
- 15e. Residue levels were not affected by applications made with either concentrated (~50 gal/A) or diluted (~250-350 gal/A) spray volumes. Residues of prohexadione-calcium were 0.142-2.631 ppm and <0.05-1.074 ppm in/on apples harvested 45 days following treatment at 1x the maximum proposed seasonal application rate in concentrated and dilute spray volumes, respectively.

OPPTS GLN 860.1520: Processed Food/Feed

Apple:

- 16a. The apple processing data are adequate for the purposes of this petition. No concentration of prohexadione-calcium residues was observed in wet pomace and juice processed from apples bearing detectable residues. Based on the results of the current processing study, tolerances for residues of prohexadione-calcium in the processed commodities of apples are not required.
- 16b. Residues of the oxidative metabolite BX 112-I5 of prohexadione, which was detected in the apple metabolism study, were less than the LOQ (<0.05 ppm) in all untreated and treated samples of whole unwashed apples. Because detectable residues were not observed in the RAC treated at the exaggerated rate (10x), the processed apple fractions were not analyzed for residues of BX 112-I5. Based on these data, the petitioner concludes that BX 112-I5 is not a metabolite of concern in apples.

Peanuts:

17. The peanut processing data are adequate for the purposes of this petition. No concentration of prohexadione-calcium residues was observed in meal and refined oil processed from peanuts bearing detectable residues. Based on the results of the current processing study, tolerances for residues of prohexadione-calcium in the processed commodities of peanuts are not required.

OPPTS GLN 860.1480: Meat, Milk, Poultry, Eggs

Ruminants:

- 18a. The cattle feeding study is acceptable. Dairy cows were orally dosed once daily with prohexadione-calcium at 8, 24, or 80 ppm (4x, 12x, and 40x, respectively, the anticipated maximum dietary burden of 2.0 ppm for dairy cattle and ~2x, ~7x, and ~24x, respectively, the anticipated maximum dietary burden for beef cattle) for 29 consecutive days. The results of the study suggest that residues of prohexadione-calcium are not likely to transfer to milk (including cream and skim milk), fat, liver, and muscle when the chemical is used according to the proposed use directions. However, residues of prohexadione-calcium are expected to transfer to liver and kidney. By extrapolation of the average residues at ~2x, ~7x, and ~24x the anticipated maximum dietary burden for beef cattle, HED concludes that the proposed tolerance levels are 0.1 ppm for residues of prohexadione-calcium in cattle kidney and 0.05 ppm for meat byproducts (except kidney). **The petitioner is required to submit a revised Section F to amend the proposed tolerance from "cattle, meat byproduct (kidney)"**

to "cattle, goats, hogs, horses, and sheep, kidney" and to add "cattle, goats, hogs, horses, and sheep, meat byproducts, except kidney."

- 18b. Cow's kidney and liver samples were additionally analyzed for residues of despropionyl prohexadione, a metabolite identified in the kidney and liver of goats from the ruminant metabolism study. At the lowest feeding level, residues of despropionyl prohexadione in cow's kidney and liver were each nondetectable (<0.05 ppm). These data suggest that residues of the despropionyl metabolite are not likely to transfer to cow's kidney and liver when the chemical is used according to the proposed use directions.

Poultry:

19. A poultry feeding study was not submitted with the subject petition. HED tentatively concludes that there is no reasonable expectation of finite residues [§180.6(A)(3)] in eggs and poultry tissues. The HED Metabolism Assessment Review Committee will be informed of this tentative conclusion but may require a poultry feeding study depending on the outcome of its deliberation. The petitioner is further advised that should dietary burden increase due to the addition of prohexadione-calcium treated poultry feed commodities through new uses, HED may require the submission of a poultry feeding study and the establishment of tolerances to cover secondary residues in eggs and poultry tissues.

OPPTS GLN 860.1850: Confined Accumulation in Rotational Crops

- 20a. The submitted confined rotational crop study is adequate. [<sup>14</sup>C]prohexadione-calcium was applied to sandy loam soil at 0.343 lb ai/A (presumably ~1x the maximum proposed seasonal application rate for peanuts). Representatives of small grains (wheat), leafy vegetables (lettuce), and root crops (turnips) were planted 31- and 122-days after treatments (DAT). Total radioactive residues were below <0.01 ppm in/on all rotational crop commodities except in 31-DAT wheat grain (0.0141 ppm), 31-DAT wheat straw (0.0237 ppm), and 122-DAT wheat straw (0.0188 ppm).
- 20b. Rotational crop commodities with total radioactivity greater than 0.01 ppm were subjected to extraction and hydrolysis procedures in order to characterize/identify residues. No residues of prohexadione-calcium or related metabolites were identified. Enzyme hydrolyses demonstrated that the majority of the radioactivity was associated with carbohydrates (120% TRR, 0.017 ppm) in wheat grain, and either carbohydrate associated (27.9% TRR, 0.007 ppm) or nonextractable (37.2% TRR, 0.009 ppm) residues in wheat straw. All extracts other

than those characterized by enzyme hydrolysis, and nonextractable residues were  $\leq 0.01$  ppm and did not require additional characterization/identification.

20c. Based on the results of this study, HED concludes that limited rotational field studies are not required and a 30-day plantback restriction is appropriate for the purpose of this petition.

#### Codex Issues

21. There is neither a Codex proposal, nor Canadian or Mexican limits for residues of prohexadione-calcium in/on plant or animal commodities. Therefore, no compatibility issues exist with regard to the proposed U.S. tolerances discussed in this petition review. A copy of the IRLS (International Residue Limit Status) sheet is attached to this memorandum.

#### RECOMMENDATIONS

HED concludes there are no residue chemistry data requirements that would preclude the establishment of permanent tolerances for residues of prohexadione-calcium in/on pome fruit, peanut and animal RACs provided that: 1) Sections B and F are revised as specified in Conclusions 2, 14, 15a and 18a; 2) prohexadione-calcium per se is determined to be the only residue of concern by the HED MARC; and 3) Agency validation of analytical enforcement method for plants is successful. Registration of Apogee™ and Baseline™ should be made conditional upon the submission of additional storage stability data as specified in Conclusions 12c and 12d. A human-health risk assessment will be prepared as a separate document.

## DETAILED CONSIDERATIONS

### OPPTS 830 Series GLNs: Product Properties

The review of product chemistry data associated with this petition is under the purview of RD. The following data for prohexadione-calcium were provided to HED in a Memo dated 5/18/99 (H. Podall, D253852):

CAS Registry No.: 127277-53-6  
Empirical Formula:  $C_{10}H_{10}O_5Ca$   
Molecular Weight: 250.27  
Vapor Pressure:  $1.00 \times 10^{-7}$  torr at 20 C (for 96.6% pure sample)  
Solubility: Solubility at 20 C for 96.6% pure sample  
174 mg/l in distilled water, 1602 mg/l in pH 5 buffer, 786 mg/l in pH 7 buffer, and 665 mg/l in pH 9 buffer  
0.038 mg/l in acetone  
0.004 mg/l in toluene  
1.11 mg/l in methanol  
0.105 mg/l in isopropanol  
<0.003 mg/l in n-hexane

The manufacturing process for technical grade prohexadione-calcium has been described. There are no impurities present which are expected to cause residue concerns.

### OPPTS GLN 860.1200: Proposed Uses

The petitioner provided a specimen label for the 27.5% dry flowable (DF) formulation [Product Name = Apogee™; EPA File Symbol No. 63588-RR] containing prohexadione-calcium as the active ingredient, and which is proposed for use on pome fruits. The 27.5% DF formulation is proposed for single, split, or multiple foliar applications to apple and pear trees at 0.206-0.825 lb ai/A/application with a maximum seasonal application rate of 1.70 lb ai/A/year. Application rates are based on a standard dilution rate of 400 gallons of water/A dilute spray using ground equipment. The first application is to be made when shoots have 1-3 inches of new growth. Retreatment intervals of 7 to 17 days are recommended; however, application of more than 0.825 lb ai/A within any 21-day interval is prohibited. A 45-day PHI is proposed. The label states that the 27.5% DF formulation is compatible with many fungicides and insecticides and may be tank mixed provided small-scale testing of the tank mix demonstrates that it is not phytotoxic. A restricted entry interval (REI) of 12 hours is proposed.



The petitioner provided a specimen label for the 75% dry flowable (DF) formulation [Product Name = Baseline™; EPA File Symbol No. 63588-O] containing prohexadione-calcium as the active ingredient, and which is proposed for use on peanuts. The 75% DF formulation is proposed for a maximum of three broadcast foliar applications at 0.125 lb ai/A/application with a 2-4 week retreatment interval using ground equipment. Each application should include 1 quart/A each of crop oil concentrate and UAN (30%). A maximum seasonal rate of 0.375 lb ai/A and a 25-day PHI are proposed. Rotational crop restrictions were not included on the Baseline™ label. However, HED has concluded that a 30-day plantback restriction is appropriate for the purpose of this petition (see section OPPTS GLN 860.1850: Confined Accumulation in Rotational Crops, below).

### Conclusions

The proposed uses of prohexadione-calcium on apples and pear (the representative commodities of pome fruits) were adequately described on the Apogee™ label. However, a revised Baseline™ label, which includes a 30-day plantback restriction for all rotational crops (except peanuts), should be submitted.

### OPPTS GLN 860.1300: Nature of the Residue in Plants

#### Apple

BASF has submitted data from a study (citation listed below) investigating the metabolism of [<sup>14</sup>C]prohexadione-calcium in apples. The in-life and analytical phases of the study were conducted by BASF (in-life phase at Holly Springs, NC, and analytical phase at Research Triangle Park, NC); however the hydrolysis of nonextractable residues was conducted by Battelle (Columbus, OH).

44725216 Jayanti, P.; Singh, M.; Oswald, J. (1998) Metabolism of (carbon-14)-BAS 125 W in Apples: Lab Project Number: 95086: 97/5005: N002762B. Unpublished study prepared by BASF Corp.

The radioactive test substance, [<sup>14</sup>C]prohexadione-calcium labeled at the C-3 and C-5 positions of the cyclohexenone ring (specific activity 18.3 mCi/mM, radiochemical purity 98.7%) was formulated with unlabeled prohexadione-calcium and an adjuvant. The formulated test substance was applied directly to apples as two run-off spray applications at ~0.88 lb ai/A/application for a total application rate of 1.76 lb ai/A (~1x the maximum proposed seasonal rate). Applications were made 35 days apart using a spray bottle. The petitioner noted that because the test substance was applied directly to apples rather than as a foliar spray to the tree, the effective application rate for apples was several orders of magnitude greater than would be expected under the proposed use.

Whole apples were collected immediately after the first application, immediately before and after the second application, and at maturity, 45 days after the second application. Samples were stored frozen (~-15 C) until analysis.

#### Total radioactive residues (TRR)

The petitioner determined TRR values for apples by summing extractable and nonextractable residues following initial aqueous extractions. Apples were extracted with basic (0.01 N NaOH water (post-application #1 and post-application #2 samples) or neutral water (pre-application #2 and 45-DAT samples); aqueous extracts were analyzed directly by LSC, and nonextractable residues were analyzed by combustion/LSC. The TRR in apples are presented in Table 1; the LOQ for TRR determinations was not reported.

Table 1. TRR in samples of apples treated with [<sup>14</sup>C]prohexadione-calcium at a total application rate of ~1.76 lb ai/A (~1x the maximum proposed seasonal rate).

| Commodity                          | TRR, ppm [ <sup>14</sup> C]prohexadione-calcium equivalents |
|------------------------------------|---|
| Apples - post-application #1       | 0.632   |
| - pre-application #2               | 0.323   |
| - post-application #2              | 0.643 (average of two extractions)                          |
| -45 days after treatment (harvest) | 0.305   |

#### Extraction of residues

Apples were subjected to extraction procedures for residue characterization and identification. During the fractionation procedures, aliquots of extracts and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. The general extraction procedures are summarized below. The distribution of radioactivity in the extracts and hydrolysates of apples is presented in Table 2.

Apples collected post-application #1 and post-application #2 were homogenized with 0.01 N NaOH (2x) and centrifuged. The resulting supernatants were filtered then combined and acidified to pH <3 with 2 N HCl. The acidified extract was partitioned with ethyl acetate (3x), and the resulting organic phase was reserved for HPLC analysis.

Apples collected pre-application #2 and 45-DAT (harvest) were homogenized with water (3x) and centrifuged. The resulting supernatants were filtered then combined and acidified to pH <1 with concentrated HCl. The acidified extract was applied to an ENV<sup>®</sup> SPE column; residues were eluted with methanol. The methanol eluate was concentrated and reserved for HPLC analysis.

The petitioner validated the extraction procedures using apples fortified with [<sup>14</sup>C]prohexadione. [<sup>14</sup>C]Prohexadione was found to be stable during extraction, methanol elution, concentration, and HPLC analysis; recoveries ranged 93.1-95.6%.

The distribution of <sup>14</sup>C-activity in the extracts and hydrolysates of apples commodities is presented in Table 2.

#### Characterization/identification of residues

The methanol extracts of apples following ethyl acetate or SPE partitioning were analyzed by HPLC for residues of prohexadione-calcium and metabolites. HPLC analysis was conducted using a Hamilton PRP-1 column and a gradient mobile phase of water acidified with formic acid (999:1, v:v) and acetonitrile (ACN) acidified with formic acid (999:1, v:v). Preparative HPLC for metabolite identification was conducted on a similar system, and HPLC analysis of methylated fractions was conducted using a Phenomenex Ultracarb 5 ODS column and a similar mobile phase. Metabolites were identified by HPLC (retention time comparison and/or co-chromatography with reference standards) and/or MS analysis including electrospray LC/MS, LC/MS/MS, and LC/MS/MS/MS and GC/MS (ionizations: CI-CH<sub>4</sub>, CI-NH<sub>3</sub>, and EI). In addition to prohexadione, the following reference standards and their methyl esters were used for retention time comparisons or co-chromatography: BX 112 I5, BX 112 M10, despropionyl prohexadione, tricarballic acid (TCA), and citric acid (refer to Figure 1 for chemical names and structures of identified metabolites).

In the organosoluble phase of post-application #1 apples, only prohexadione was identified by HPLC; in post-application #2 apples, prohexadione was the major component along with minor levels of weathered residues. In the organosoluble phases of pre-application #2 and 45-DAT (harvest) apples, initial HPLC analysis showed about 21 peaks; the majority of unknowns detected were determined to be more polar than the parent.

To identify the metabolites of prohexadione in apples, several batches of pre-application #2 and 45-DAT apples were extracted with water; the extracts were applied to ENV SPE columns, and residues were eluted with ACN, tetrahydrofuran, or 2% formic acid:ACN. The purified fractions were then subjected to preparative HPLC. Following HPLC separation, fractions collected for a single peak (based on retention time) from various extraction batches were pooled. The pooled fractions were then subjected to HPLC analysis. The seven major fractions, 6F, 19F, 21F, 25F, 27F, 31F, and 45F, were concentrated under reduced pressure to remove solvent, and were subjected to either lyophilization and reconstitution in 0.1% formic acid:ACN (Fractions 6F, 19F, and 21F) or acidification to pH 1.5 with concentrated HCl followed by purification on an HLB SPE

column eluted with 1% formic acid:ACN (Fractions 25F, 27F, 31F, and 45F).

To facilitate identification of metabolites, isolated fractions were subjected to methylation, either before or after preparative HPLC, with diazomethane; Fraction 31F was also methylated with methanol:sulfuric acid to produce a dimethylated product. The methylated samples were purified by HPLC and subjected to HPLC co-chromatography with methylated reference standards. Identification of individual metabolites in the seven major fractions is described below.

Identification of prohexadione in fraction 45F was confirmed by HPLC co-chromatography, MS analysis, and negative ion LC/MS, LC/MS/MS, and LC/MS/MS/MS analysis. A second sub-fraction of 45F, fraction 45F2-A, was found to be identical to metabolite 27F2-B (see below) following methylation and subsequent LC/MS/MS and GC/MS analysis.

Identification of tricarballic acid (TCA) in Fraction 6F was confirmed by initial retention time comparison of the peak in the non-methylated sample and by co-chromatography of the methylated fraction with TCA-methylester. Dimethyl despropionyl prohexadione, an apparent intermediate, was tentatively identified in Fraction 6F by retention time comparison.

Identification of citric acid in Fraction 19F was confirmed by retention time comparisons and co-chromatography with citric acid-methylester in the methylated fraction; dimethyl-despropionyl prohexadione was tentatively identified by retention time comparison.

Identification of dimethyl-despropionyl prohexadione and BX-112-M10 was confirmed in Fraction 21F by retention time comparisons and co-chromatography in the methylated fraction.

Preparative HPLC of fraction 25F produced four fractions, 25F1, 25F2, 25F3, and 25F4. When the four fractions were methylated with diazomethane, fractions 25F1, 25F2, and 25F3 yielded a single product based on retention times and were combined to form fraction 25F1-A; fraction 25F4 was determined to consist of several minor products and was not investigated further. LC/MS/MS and GC/MS analysis were used to develop a putative structure for the dihydroxy metabolite 25F1-A.

Preparative HPLC of fraction 27F produced fractions 27F1 and 27F2, which were in turn methylated to produce fractions 27F1-A, 27F2-A and 27F2-B. The petitioner concluded that fraction 27F1-A was identical to 27F2-A. LC/MS/MS and GC/MS analysis were used to develop structures for fractions 27F1-A, the diacid metabolite, and 27F2-B, the benzoic acid derivative.

Identification of BX-112-15 in Fraction 31F was achieved by LC/MS/MS, GC/MS, HPLC co-chromatography with a non-methylated standard, and GC/MSD co-injection of the monomethylated derivative with a BX-112-15 methylester standard.

The aqueous-soluble fraction from 45-DAT apples (Aqueous 2) was freeze dried and subjected to HPLC analysis on a Phenomenex Ultracarb 5 ODS column. Most of the radioactivity eluted at the solvent front. HPLC analysis following methylation indicated six different, highly polar components.

An aliquot of nonextractable residues from the 45-DAT apples was subjected to base extraction with 1 N NaOH (at ambient temperature for 16 hours); the hydrolysate was neutralized with HCl and filtered through a molecular weight filtration apparatus (3000 MW), then adjusted to pH 3 with formic acid and reserved for HPLC analysis. A separate aliquot of the nonextractable residues was subjected to acid hydrolysis with 72% sulfuric acid (bubbled with argon at 30 C for 45 minutes) followed by 3% sulfuric acid (bubbled with argon at 127.5 C, 22 psi, for 1 hour). The hydrolysate was adjusted to pH 7 with calcium carbonate and centrifuged, and the supernatant was derivatized with phenylhydrazine hydrochloride (in sodium acetate buffer at 95 C for 1 hour) and refrigerated to induce osazone crystallization. The resulting precipitate was dissolved in hot ethanol and reserved for TLC analysis; however, TLC analysis was unsuccessful. The petitioner concluded that any osazones formed did not elute from the TLC origin.

A summary of the characterized and identified <sup>14</sup>C-residues in apples is presented in Table 3. The chemical structures and chemical names for identified metabolites are presented in Figure 1.

Table 2. Distribution and characterization of radioactive residues in apples treated with [<sup>14</sup>C]prohexadione-calcium at a total application rate of 1.76 lb ai/A (~1x the maximum proposed seasonal rate).

| Fraction   | % TRR | ppm   | Characterization/Identification <sup>A</sup>  |
|--|-------|-------|---|
| <b>Apple - Post Application #1 (TRR = 0.632 ppm)</b> |       |       |   |
| Aqueous 1  | 92.81 | 0.586 | Partitioned with EtOAc.   |
| Organosoluble  | 81.30 | 0.514 | HPLC analysis resolved prohexadione based on retention time comparison with a standard; quantitative results not reported.  |
| Aqueous 2  | 11.87 | 0.075 | Not further analyzed (N/A).   |
| Nonextractable                                       | 7.19  | 0.045 | N/A.  |
| <b>Apple - Preapplication #2 (TRR = 0.323 ppm)</b>   |       |       |   |
| Aqueous 1  | 92.54 | 0.299 | Subjected to SPE; residues were eluted with methanol.   |
| Organosoluble  | 74.28 | 0.240 | HPLC analysis resolved 21 peaks (based on retention time) ranging from 1.12% to 9.55% TRR. The peaks corresponded to peaks resolved in 45-DAT apples.   |
| Aqueous 2  | 10.08 | 0.033 | N/A.  |
| Nonextractable                                       | 7.46  | 0.024 | N/A.  |
| <b>Apple - Post Application #2 (TRR = 0.536 ppm)</b> |       |       |   |
| Aqueous 1  | 94.34 | 0.506 | Partitioned with EtOAc.   |
| Organosoluble  | 60.76 | 0.326 | HPLC analysis resolved mainly prohexadione along with some weathered metabolites; quantitative results not reported.  |
| Aqueous 2  | 24.98 | 0.134 | N/A.  |
| Nonextractable                                       | 5.66  | 0.030 | N/A.  |
| <b>Apple - 45-DAT (TRR = 0.305 ppm)</b>              |       |       |   |
| Aqueous 1  | 92.42 | 0.282 | Subjected to SPE; residues were eluted with methanol.   |
| Organosoluble  | 83.40 | 0.254 | <u>HPLC analysis resolved:</u><br>Prohexadione 1.72% TRR 0.0052 ppm<br>BX 112-IS 11.78% TRR 0.0359 ppm<br>BX 112-M10 9.21% TRR 0.0281 ppm<br>Despropionyl 5.55% TRR 0.0169 ppm<br>Metabolite 27F2-B 7.68% TRR 0.0234 ppm<br>Metabolite 25F1-A 5.33% TRR 0.0163 ppm<br>Metabolite 27F2-A 2.58% TRR 0.0079 ppm<br>TCA 0.79% TRR 0.0024 ppm<br>Citric acid 2.44% TRR 0.0074 ppm<br>Unknown 6F1 (polar) 0.59% TRR 0.0018 ppm<br>Unknown 9F (polar) 4.40% TRR 0.0134 ppm<br>Unknown 13F (polar) 3.60% TRR 0.0110 ppm<br>Peak 5 1.86% TRR 0.0057 ppm<br>Unknowns 19F1, 19F2, 19F4 5.46% TRR 0.0166 ppm<br>Unknown 25F4 (4 components) 1.43% TRR 0.0044 ppm<br>Peak 12 2.28% TRR 0.0070 ppm<br>Unknown 33F 5.49% TRR 0.0167 ppm<br>Unknown 35F (5 components) 6.22% TRR 0.0190 ppm<br>Peak 17 1.88% TRR 0.0057 ppm<br>Peak 18 3.11% TRR 0.0095 ppm |

Table 2 (continued).

| Fraction                    | % TRR            | ppm   | Characterization/Identification <sup>A</sup>  |
|-----------------------------|------------------|-------|---|
| Aqueous 2                   | 5.92             | 0.018 | Following methylation, HPLC analysis resolved 6 different, highly polar components  |
| Nonextractable              | 7.58             | 0.023 | Separately subjected to: (I) base hydrolysis with 1 N NaOH and concentrated; and (ii) acid hydrolysis   |
| (I) Base hydrolysate        | 2.96             | 0.009 | Subjected to molecular weight filtration.   |
| <3000 MW                    | 1.87             | 0.006 | <u>HPLC analysis resolved:</u><br>Prohexadione 0.11% TRR 0.0003 ppm<br>TCA 0.45% TRR 0.0014 ppm<br>Plus 5 additional unknowns, each present at $\leq 0.50\%$ TRR, totaling 1.32% TRR (0.0040 ppm) |
| >3000 MW                    | 1.29             | 0.004 | Fraction determined to consist of macromolecules and cellular components.   |
| Nonextractable              | N/R <sup>b</sup> | N/R   | N/A.  |
| (ii) Acid hydrolysate       | 2.2              | 0.007 | Subjected to derivatization with phenylhydrazine to produce osazone crystals; fraction characterized by the petitioner as glucose osazone.  |
| Derivative supernatant      | 1.4              | 0.004 | N/A.  |
| Precipitate                 | 0.6              | 0.002 | Dissolved and subjected to TLC analysis; any osazones formed did not elute from the TLC origin.   |
| Nonextractable <sup>c</sup> | 2.21             | 0.074 | N/A.  |

<sup>A</sup> See Figure 1 for names and structures of identified metabolites.

<sup>b</sup> N/R = Not reported.

<sup>c</sup> Calculated by difference by the petitioner.

Table 3. Summary of radioactive residues characterized/identified in apples treated with [<sup>14</sup>C]prohexadione-calcium at a total application rate of 1.76 lb ai/A (~1x the maximum proposed seasonal rate).

| Fraction  | Apple - 45-DAT (TRR = 0.305 ppm) |               |
|---|----------------------------------|---------------|
|   | % TRR                            | ppm           |
| <b>Identified <sup>a</sup></b>                          |                                  |               |
| Prohexadione  | 1.83                             | 0.0056        |
| BX 112-I5   | 11.78                            | 0.0359        |
| BX 112-M10  | 9.21                             | 0.0281        |
| Despropionyl prohexadione                               | 5.55                             | 0.0169        |
| 27F2-B (and 45F2-A)                                     | 7.68                             | 0.0234        |
| 25F1-A  | 5.33                             | 0.0163        |
| 27F2-A (and 27F1-A)                                     | 2.60                             | 0.0079        |
| TCA   | 1.24                             | 0.0038        |
| Citric acid   | 2.44                             | 0.0074        |
| <b>Total identified</b>                                 | <b>47.66</b>                     | <b>0.1453</b> |
| <b>Characterized</b>                                    |                                  |               |
| Unknown 6F1 (polar)                                     | 0.59                             | 0.0018        |
| Unknown 9F (polar)                                      | 4.40                             | 0.0134        |
| Unknown 13F (polar)                                     | 3.60                             | 0.0110        |
| Peak 5  | 1.86                             | 0.0057        |
| Unknowns 19F1, 19F2, 19F4                               | 5.46                             | 0.0166        |
| Unknown 25F4 (4 components)                             | 1.43                             | 0.0044        |
| Peak 12   | 2.28                             | 0.0070        |
| Unknown 33F   | 5.49                             | 0.0167        |
| Unknown 35F (5 components)                              | 6.22                             | 0.0190        |
| Peak 17   | 1.88                             | 0.0057        |
| Peak 18   | 3.11                             | 0.0095        |
| Aqueous soluble unknowns (6)                            | 5.92                             | 0.0181        |
| Base hydrolysate unknowns <3000 MW (5, each <0.50% TRR) | 1.32                             | 0.0040        |
| Base hydrolysate unknowns >3000 MW                      | 1.29                             | 0.0039        |
| Glucose osazones  | 2.20                             | 0.0067        |
| <b>Total identified/characterized</b>                   | <b>94.71</b>                     | <b>0.2888</b> |
| Nonextractable <sup>b</sup>                             | 2.21                             | 0.0067        |

<sup>a</sup> See Figure 1 for names and structures of identified metabolites.

<sup>b</sup> Calculated by difference by the petitioner.



### Storage stability

Samples and extracts from the apple metabolism study were stored frozen for up to 735 days (24.2 months) until final HPLC analysis for quantitation of residues. To demonstrate the stability of prohexadione and metabolites in apples, the petitioner conducted a storage stability study with a representative 45-DAT sample analyzed concurrently with major experimental milestones. HPLC metabolite profiles for initial aqueous extracts and organosoluble fractions and extraction results were compared for analyses conducted 4 months after sample collection and 24 months after collection. The data indicated that the metabolite profiles in terms of relative ratios for individual peaks and the presence of individual metabolites remained stable over the study interval as did the extractability of radioactive residues. These data are adequate to support the subject apple metabolism study.

### Study summary

The apple metabolism study is acceptable. TRRs were 0.305 ppm in/on mature apples harvested 45 days following the last of two sequential applications of [<sup>14</sup>C]prohexadione-calcium, labeled at the C-3 and C-5 positions of the cyclohexenone ring, at a total application rate of 1.76 lb ai/A (~1x the maximum proposed seasonal rate). Applications were made directly to apples as a run-off spray.

Over 94% of the TRR in/on mature apples were characterized and identified. The characterization/identification of the organosoluble fractions indicated that the radioactive residues consisted of several minor components, none greater than 12% of the TRR. Prohexadione-calcium was rapidly metabolized to prohexadione (free acid), which was identified at 1.83% TRR (0.0056 ppm). Metabolites BX 112-I5 and BX 112-M10 were the major identified metabolites, accounting for 11.78% TRR (0.0359 ppm) and 9.21% TRR (0.0281 ppm), respectively. The following metabolites were also identified: despropionyl prohexadione (5.55% TRR, 0.0169 ppm), 27F2-B (7.68% TRR, 0.0234 ppm), 25F1-A (5.33% TRR, 0.0163 ppm), 27F2-A (2.60% TRR, 0.0079 ppm), tricarballic acid (1.24% TRR, 0.0038 ppm), and citric acid (2.44% TRR, 0.0074 ppm). Organosoluble unknowns (20 components) accounted for 36.32% TRR and aqueous-soluble unknowns (6 components) accounted for 5.92% TRR. The petitioner demonstrated that the remainder of the characterized radioactivity was distributed between base hydrolysate unknowns <3000 MW (5 components) at 1.32% TRR, base hydrolysate unknowns >3000 MW at 1.29% TRR, and glucose osazones at 2.20% TRR.

## Peanuts

BASF has submitted data from a study (citation listed below) investigating the metabolism of [<sup>14</sup>C]prohexadione-calcium in peanuts. The in-life and analytical phases of the study were conducted by BASF (in-life phase at Holly Springs, NC, and analytical phase at Research Triangle Park, NC).

44457793 Steginsky, C.; Powell, J.; Winkler, V. et al. (1997) Metabolism of (carbon 14)-BAS 125 W (Prohexadione Calcium) in Peanut: Lab Project Number: M9412: 97/5341: SC930242. Unpublished study prepared by Battelle and Agvise Labs., Inc.

The radioactive test substance, [<sup>14</sup>C]prohexadione-calcium labeled at the C-3 and C-5 positions of the cyclohexenone ring (specific activity 15.3 mCi/mM, radiochemical purity 98.6%) was formulated with an equal amount of [<sup>13</sup>C]prohexadione-calcium. Water and an adjuvant were added to yield a final specific activity of 63,700 dpm/μg. The formulated test substance was applied, using a manual sprayer, to peanuts grown in an outdoor plot as an over-the-top postemergence spray at ~1.0 lb ai/A (~2.7x the maximum proposed seasonal rate of 0.375 lb ai/A). Plants in the control plot were not treated.

Peanut plant samples were collected on the day of treatment and 13 and 22 days after treatment (DAT). The 22-DAT (harvest) samples were allowed to dry in the field for 3 days, and were separated into pods and vines in the field. The pods and vines/hay were then further dried under laboratory hoods to achieve a moisture content of 10-15% to simulate field practices. The hood-dried whole peanuts were then separated into nutmeats and hulls. Following harvest and/or drying as appropriate, samples were stored at ~-20C.

## Total radioactive residues (TRR)

Triplicate aliquots of peanut nutmeats, hulls, and hay, were homogenized with dry ice and subjected to combustion/LSC for TRR determinations. The TRR in peanut commodities are presented in Table 4; the reported LOD for TRR determinations was 0.001 ppm.

Table 4. Total radioactive residues in samples of peanut commodities treated with [ $^{14}\text{C}$ ]prohexadione-calcium at a total application rate of ~1 lb ai/A (~2.7x the maximum proposed seasonal rate).

| Commodity                | TRR, ppm [ $^{14}\text{C}$ ]prohexadione-calcium equivalents |
|--------------------------|--|
| 0-DAT <sup>A</sup> Vines | 634.6  |
| 13-DAT Vines             | 19.8   |
| 22-DAT Vines (not dried) | 18.3   |
| 22-DAT Nutmeats          | 4.15   |
| 22-DAT Hulls             | 2.50   |
| 22-DAT Hay               | 36.5   |

<sup>A</sup> DAT = days after treatment.

#### Extraction and hydrolysis of residues

Homogenized samples of nutmeats, hulls, and hay were subjected to extraction and hydrolysis procedures for residue characterization and identification. During the fractionation procedures, aliquots of extracts, hydrolysates, and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. The general extraction procedures are summarized below.

Subsamples of nutmeats, hulls, and hay were extracted 3-4x with acetonitrile (ACN):1.5 M sulfuric acid (9:1, v:v), then centrifuged. Hulls were initially hydrated overnight prior to extraction. The supernatants were pooled and concentrated by rotary evaporation to remove solvent. The concentrated extracts were then partitioned 3x with dichloromethane (DCM):ACN (3:1, v:v). After each partition, the phases were allowed to separate. The respective DCM phases and aqueous phases for each matrix were pooled; the pooled DCM fractions were concentrated and reserved for HPLC analysis, and the pooled aqueous fractions were adjusted to pH 3 with 6 N NaOH and reserved for HPLC analysis.

Nonextractable residues following initial extraction were extracted 2x with hexane then centrifuged. The hexane supernatants were pooled, and the nonextractable residue were dried. To solubilize oily residues, the dried nonextractable residues from nutmeats were extracted 2x with petroleum ether:ether (87:13, v:v), then centrifuged. The ether supernatants were pooled.

The petitioner validated the extraction procedures using untreated peanut hay fortified with [ $^{14}\text{C}$ ]prohexadione-calcium. When the fortified sample was extracted shortly after fortification, approximately 90.8% of the TRR in the fortified sample was extracted into ACN:sulfuric acid, ~0.01% TRR into the hexane extract, and 5.59% TRR remained in the nonextractable residues. When the ACN:sulfuric acid extract was partitioned with DCM:ACN, ~83.5% TRR partitioned into DCM, and ~1.5% TRR partitioned into the

aqueous fraction. HPLC analysis of the DCM fraction revealed a single peak (prohexadione) accounting for 81.9% TRR.

To evaluate residue dissipation in peanut vines, subsamples of 0-DAT, 13-DAT and 22-DAT vines were extracted with ACN:sulfuric acid as described above, and analyzed by HPLC. The concentration of prohexadione decreased from 79.0% TRR in 0-DAT vines to 27.2% TRR in 13-DAT vines, and to 24.0% TRR in 22-DAT vines. The concentrations of polar metabolites and nonextractable residues each increased to ~30% TRR in 22-DAT vines.

The distribution of  $^{14}\text{C}$ -activity in the extracts and hydrolysates of peanut commodities is presented in Table 5.

#### Characterization/identification of residues

The extracts of peanut nutmeats, hulls, and hay were analyzed by HPLC for residues of prohexadione-calcium and metabolites. HPLC analyses were conducted using Hamilton PRP-1 or YMC ODS-Aq columns, UV (254, 235, 210, or 205 nm) or radioflow detectors, and gradient mobile phases of 0.1% acetic acid in deionized water:0.1% acetic acid in ACN. In addition, isolated components from the DCM fractions were analyzed by one-dimensional TLC on silica gel F<sub>254</sub> plates developed in isopropyl ether:formic acid:deionized water (90:7:3, v:v:v). Radioactivity was detected and quantified using a radioanalytic imaging system; nonlabeled standards were visualized using UV light. Preparative HPLC for metabolite identification was conducted on systems similar to those described above, and HPLC analysis of methylated fractions was conducted using a Phenomenex Ultracarb 5 ODS column and a similar mobile phase. Metabolites were quantitated by fraction collection and preparative HPLC following additional partitioning and/or filtration procedures. The petitioner stated that fluctuations were observed in retention times for standards resulting from different manufacturing lots for columns, low flow rates, flow gradients, and fraction collection procedures. Consequently, characterization of radioactive peaks was accomplished by co-chromatography with standards or with isolated radioactive peaks rather than by retention time comparisons. For certain components, MS and/or NMR analyses were used for identification. The following reference standards were used for co-chromatography: prohexadione-calcium, prohexadione, prohexadione methylester, despropionyl prohexadione (and its methylated and dimethylated esters), dioxopropyl prohexadione, tricarballic acid (TCA), and glutaric acid.

Subsamples of hay were subjected to bulk extraction and clean-up procedures for use in characterization of metabolites. The subsamples were extracted according to the procedure described above except that nonextractable residues were not extracted with hexane. The DCM fraction was concentrated, diluted with deionized

water, passed through a 3000 MW filter, then centrifuged. The <3000 MW fraction was concentrated by rotary evaporation and subjected to semi-preparative HPLC.

The aqueous fraction of hay following bulk extraction was diluted with deionized water and applied to a C18 column. Residues were eluted with 0.1% acetic acid in deionized water:0.1% acetic acid in ACN (70:30, v:v), and the eluate was applied to a series of strong anion exchange solid-phase extraction (SAX/SPE) columns. Residues were eluted with 2 N HCl in ACN. The eluates were subjected to semi-preparative HPLC.

Hay: Prohexadione was identified in the aqueous fraction of hay by LC/MS following isolation by preparative HPLC. Identification of prohexadione in the DCM fraction was confirmed by HPLC and TLC co-chromatography with <sup>14</sup>C-labeled and nonlabeled standard.

The region containing prohexadione from the aqueous fraction was subjected to strong acid (6 N HCl) and base (6 N NaOH) hydrolysis, each at 100 C for 1 hour. On the basis of HPLC analysis of the base hydrolysate, the petitioner concluded that minor components were mostly base-labile conjugates (probably ester type linkages) of prohexadione, with some conjugates of various polar metabolites including TCA. Acid hydrolysis resulted in degradation of prohexadione.

TCA was identified in the aqueous fraction of hay by co-chromatography following isolation by preparative HPLC. Identification was confirmed by LC/MS/MS analysis following methylation of the isolated peak with BF<sub>3</sub>:methanol.

Metabolite dioxopropyl prohexadione (HA4) was isolated from both the DCM and aqueous fractions of hay. The isolated peak from the aqueous fraction was characterized by strong acid and strong base hydrolysis as described above, followed by HPLC. Dioxopropyl prohexadione was stable to acid hydrolysis, but was degraded by base hydrolysis. The petitioner noted that the elution behavior of the base hydrolyzed degradate suggested an open chain organic acid like TCA. The isolated component from the DCM fraction, was stable when subjected to enzymatic hydrolysis with  $\alpha$ -glucosidase (in 100 mM potassium phosphate buffer, pH 7, at 37 C for 21.5 hours) and  $\beta$ -glucosidase (in 100 mM acetate buffer, pH 5, at 37 C for 21.5 hours). The structure of dioxopropyl prohexadione was elucidated by LC/MS of the isolated peak and by separate LC/MS/MS analyses of the component following methylation with H<sub>2</sub>SO<sub>4</sub>:methanol and BF<sub>3</sub>:methanol. Identification was confirmed by <sup>13</sup>C- and <sup>1</sup>H-NMR analysis of the isolated peak from peanut vine.

Metabolites HA5 and HA6 were isolated from the DCM fraction of hay, and were separately characterized by mild base hydrolysis with 0.5 N NaOH (at 40 C, overnight) and enzyme hydrolysis with  $\alpha$ - and  $\beta$ -

glucosidase. Both metabolites were hydrolyzed to dioxopropyl prohexadione under mild base conditions, and were apparently interconverted during enzyme hydrolysis. The petitioner confirmed that the interconversion between HA5 and HA6 resulted from incubation with acetate buffer and not from enzyme hydrolysis. Although no structures were proposed for metabolites HA5 and HA6, the petitioner concluded, based on the results of these characterization procedures that these are oxidation products similar to dioxopropyl prohexadione.

**Nutmeats:** Prohexadione was identified in the DCM fraction of nutmeats by HPLC and TLC co-chromatography with labeled and non-labeled standard following isolation by preparative HPLC. Despropionyl prohexadione was identified in the aqueous fraction of peanut nutmeats following base hydrolysis by HPLC co-chromatography of the base hydrolysate with unlabeled standard. Prohexadione, TCA, dioxopropyl prohexadione, HA5, and HA6 were identified in the aqueous fractions of nutmeats by co-chromatography with the hay aqueous fraction.

Determination of metabolites HA5 and HA6 in the DCM fraction of peanut nutmeats was confirmed by co-chromatography with the corresponding peaks from the hay DCM fraction and by preparative HPLC followed by retention time comparisons. Following mild base hydrolysis, peaks HA5 and HA6 in peanut nutmeats hydrolyzed to dioxopropyl prohexadione as they did in hay.

The results of HPLC analysis following strong acid and base hydrolysis of the aqueous fraction of nutmeats were similar to those for the aqueous fraction of hay; however, because the major hydrolysis products in nutmeat were despropionyl prohexadione and prohexadione, the petitioner concluded that minor components in the aqueous fraction were base-labile conjugates of despropionyl prohexadione or prohexadione.

**Hulls:** Characterization of residues in hulls was achieved using the procedures described above. Prohexadione was identified in the DCM fraction by HPLC and TLC co-chromatography. Prohexadione, TCA, and dioxopropyl prohexadione were identified in the aqueous fraction by co-chromatography of the aqueous fraction of hulls with the aqueous fraction from hay. Isolated peaks from the DCM fraction of hulls were identified as Metabolites HA5 and HA6 by co-chromatography with the corresponding peaks from hay, and minor components in the aqueous fraction were characterized as conjugates of prohexadione following strong acid and base hydrolysis.

**Nonextractable residues:** Nonextractable residues in nutmeats, hulls, and hay were subjected to the following additional characterization procedures. To solubilize cellular components such as soluble proteins, oligosaccharides, oligopeptides, and low molecular weight components which co-precipitated with cellular

components during solvent extraction, subsamples of the postextraction solids were extracted 5x with 50 mM sodium acetate buffer (pH 5) and centrifuged. The supernatants for each matrix were pooled and applied to a 3000 MW filter. The <3000 MW fraction was concentrated and analyzed by HPLC. About half of the radioactivity in nutmeats and hay and 20% of the radioactivity in hulls was solubilized by buffer extraction; the majority of the solubilized radioactivity from all matrices (80-90%) was found in the <3000 MW fraction. Prohexadione was identified in nutmeats and hay by co-chromatography with non-labeled standard. TCA was identified in hay by retention time comparison with the isolated peak from the bulk extraction of hay. TCA, dioxopropyl prohexadione, and HA5 and HA6 were identified in hulls by pattern comparison of the observed peaks in the <3000 MW fraction with the peaks in the aqueous fraction of hulls.

Aliquots of the nonextractable residues following buffer extraction were subjected to base and acid hydrolysis with 1 N NaOH (at 100 C for 1 hour) or 2 N HCl (at 100 C for 1 hour) followed by centrifugation and HPLC analysis of the supernatants. About one-third to one-half of the radioactivity in nutmeats, hulls, and hay was hydrolyzed by NaOH. Prohexadione was identified in nutmeats, hulls, and hay by co-chromatography with non-labeled standard. Despropionyl prohexadione was identified in hay and hulls and TCA was identified in hulls by retention time comparisons with nonlabeled standards. Because prohexadione was degraded by acid hydrolysis, results of this procedure were not reported.

Separate subsamples of the nonextractable residues of nutmeats, hulls, and hay were subjected to buffer extraction as described above, and the resulting nonextractable residues were subjected to carbohydrate enzyme hydrolysis with hemicellulase, amylase, cellulysin, amyloglucosidase, and pectinase (in 50 mM acetate buffer, pH 5, at 40 C for 24 hours), then centrifuged. Carbohydrate enzyme hydrolysis released ca. one-half, one-sixth, and one-fourth of the radioactivity in subsamples of nutmeats, hulls, and hay, respectively. The pellet remaining following carbohydrate enzyme hydrolysis was subjected to protein enzyme hydrolysis with pronase (in 0.05 M Tris/HCl buffer and 0.01 M calcium chloride, at 40 C for 24 hours), then centrifuged. Pronase released 1%-2% TRR in nutmeats, hulls, and hay. Hydrolysis procedures were also conducted with boiled (inactive) enzymes to determine how much of the degradation was enzyme dependent. Prohexadione was identified in the carbohydrate and protein enzyme hydrolysates of nutmeats, hulls, and hay by HPLC co-chromatography with nonlabeled standard.

A subsample of the carbohydrate enzyme hydrolysate of nutmeats was filtered through a 3000 MW filter. One aliquot of the <3000 MW fraction was subjected to mild base hydrolysis (1 N NaOH at 100 C for 1 hour). A second aliquot was concentrated to dryness and

subjected to acid hydrolysis with 72% sulfuric acid (bubbled with argon at 30 C for 45 minutes). The hydrolysate was cooled, diluted with water, neutralized with excess calcium carbonate, and centrifuged. The supernatant was derivatized with phenylhydrazine hydrochloride (in sodium acetate buffer at 95 C for 1 hour) and refrigerated to induce osazone crystallization. The resulting precipitate was dissolved in hot ethanol and recrystallized with cold water. The osazone crystals contained 2% TRR. The same derivatization procedure was also performed on the nonextractable residues of nutmeats following solvent extraction except that the recrystallization step was repeated several times. The osazone derivative contained 8.43% TRR.

To characterize lignin, the enzyme hydrolysates of hulls and hay were refluxed 4x with dioxane:2 N HCl (9:1, v:v) for 30 minutes. Following centrifugation, the supernatants for each matrix were pooled, concentrated to dryness, reconstituted in deionized water and centrifuged again. The aqueous-soluble hull-lignin phase was analyzed by HPLC. To further characterize the aqueous-soluble hay lignin fraction, an aliquot was filtered through a 3000 MW filter; ca. 75% of the fraction consisted of <3000 MW components. A second aliquot of the hay lignin aqueous soluble fraction was subjected to strong base hydrolysis as described above, then filtered through a 3000 MW filter. The <3000 MW fraction was analyzed by HPLC. Nonextractable residues (aqueous insoluble) following dioxane:HCl extraction were resolubilized in dioxane:2 N HCl (9:1, v:v), concentrated to dryness and subjected to hydrolysis with 1 N NaOH (at 100 C for 1 hour). The <3000 MW fraction was analyzed by HPLC. Prohexadione was released by base hydrolysis of the aqueous soluble and insoluble fractions, and identification confirmed by co-chromatography with unlabeled standard.

A summary of the characterized and identified <sup>14</sup>C-residues in peanut commodities is presented in Table 6.



Table 5. Distribution and characterization of radioactive residues in peanut commodities harvested 22 days following treatment with [<sup>14</sup>C]prohexadione-calcium at a total application rate of ~1 lb ai/A (~2.7x the maximum proposed seasonal rate).

| Fraction                         | % TRR            | ppm   | Characterization/Identification <sup>a</sup>  |
|----------------------------------|------------------|-------|---|
| <b>Nutmeats (TRR = 4.15 ppm)</b> |                  |       |   |
| ACN:sulfuric acid                | 63.1             | 2.62  | Partitioned with DCM:ACN  |
| DCM*                             | 34.0             | 1.41  | <u>HPLC analysis resolved:</u><br>Prohexadione 18.8% TRR 0.780 ppm<br>HA5 2.99% TRR 0.124 ppm<br>HA6 3.75% TRR 0.156 ppm<br>NMC2 1.37% TRR 0.057 ppm<br>NMC3 1.00% TRR 0.042 ppm<br>NMC4 3.88% TRR 0.161 ppm<br>Other unknowns 2.21% TRR 0.091 ppm<br>(ea <0.4% TRR)  |
| Aqueous*                         | 26.9             | 1.12  | <u>HPLC analysis resolved:</u><br>Prohexadione 3.21% TRR 0.133 ppm<br>Dioxopropyl prohexadione 0.93% TRR 0.039 ppm<br>TCA 1.58% TRR 0.066 ppm<br>HA5 2.64% TRR 0.110 ppm<br>HA6 2.26% TRR 0.094 ppm<br>NA1 2.10% TRR 0.087 ppm<br>Other unknowns 14.20% TRR 0.592 ppm<br>(ea <1.5% TRR)<br><br><u>Additional characterization of selected fractions resolved:</u><br>Polar <3000 MW 0.44% TRR 0.018 ppm<br>Base-labile conjugates 10.1% TRR 0.418 ppm |
| Nonextractable                   | N/R <sup>b</sup> | N/R   | Extracted with hexane.  |
| Hexane (lipids)*                 | 3.01             | 0.125 | Not further analyzed (N/A).   |
| Nonextractable                   | N/R              | N/R   | Extracted with petroleum ether:ether  |
| Ether*                           | 2.70             | 0.112 | <u>HPLC analysis resolved:</u><br>Prohexadione 2.47% TRR 0.103 ppm<br>Other unknowns 0.23% TRR 0.010 ppm<br>(ea <0.15% TRR)   |
| Nonextractable                   | 41.8             | 1.73  | Subjected to extraction with sodium acetate buffer followed by: (i) carbohydrate and protein enzyme hydrolysis and lignin characterization via dioxane:2 N HCl; and (ii) mild base hydrolysis/molecular weight filtration.  |
| (i) Buffer-soluble               | 25.7             | 1.06  | Characterized as cellular components.   |
| Nonextractable                   | 16.1             | 0.666 | Subjected to enzyme hydrolysis with hemicellulase, amylase, cellulysin, amyloglucosidase, and pectinase.  |
| Carbohydrate enzyme hydrolysate  | 12.6             | 0.52  | Based on comparison of results with inactivated enzymes, 10.09% TRR (0.419 ppm) characterized as carbohydrates.<br><br>Filtered through a 3000 MW filter.   |
| <3000 MW (sugars)                | 8.42             | 0.347 | Subjected to (i) mild base hydrolysis with 1 N NaOH; and (ii) derivatization with phenylhydrazine-HCl to osazones.  |

Table 5 (continued).

| Fraction                      | % TRR | ppm   | Characterization/Identification <sup>a</sup>  |
|-------------------------------|-------|-------|---|
| (i) 1 N NaOH hydrolysate *    | N/R   | N/R   | HPLC analysis resolved:<br>Prohexadione 5.13% TRR 0.211 ppm<br>Plus one additional unknown at 3.29% TRR (0.136 ppm).  |
| (ii) Osazone crystals         | 1.52  | 0.063 | N/A.  |
| >3000 MW                      | 2.76  | 0.114 | N/A.  |
| Nonextractable                | 3.53  | 0.146 | Subjected to enzyme hydrolysis with pronase.  |
| Protein hydrolysate *         | 2.33  | 0.096 | Based on comparison of results with inactivated enzymes, 0.33% TRR (0.014 ppm) characterized as protein.<br><br>HPLC analysis resolved:<br>Prohexadione 1.17% TRR 0.0481 ppm<br>Other unknowns 1.16% TRR 0.0481 ppm<br>(ea $\leq$ 0.37% TRR)                        |
| Nonextractable                | 1.82  | 0.075 | N/A.  |
| (ii) Buffer-soluble           | 22.2  | 0.918 | Filtered through 3000 MW filter.  |
| <3000 MW *                    | 18.6  | 0.767 | HPLC analysis resolved:<br>Prohexadione 7.47% TRR 0.308 ppm<br>Dioxopropyl prohexadione 1.15% TRR 0.047 ppm<br>TCA 1.48% TRR 0.061 ppm<br>Other unknowns 8.50% TRR 0.356 ppm<br>(ea $\leq$ 1% TRR)  |
| >3000 MW *                    | 3.93  | 0.162 | N/A.  |
| Nonextractable                | 19.6  | 0.811 | Subjected to mild base hydrolysis with 1 N NaOH   |
| Base hydrolysate              | 10.5  | 0.436 | HPLC analysis resolved:<br>Prohexadione 2.41% TRR 0.100 ppm<br>Plus two additional unknowns at 6.48% TRR (0.269 ppm) and 1.60% TRR (0.067 ppm)  |
| Nonextractable                | 1.10  | 0.046 | N/A.  |
| <b>Hulls (TRR = 2.50 ppm)</b> |       |       |   |
| ACN:sulfuric acid             | 56.8  | 1.42  | Partitioned with DCM:ACN  |
| DCM*                          | 8.38  | 0.210 | HPLC analysis resolved:<br>Prohexadione 2.50% TRR 0.063 ppm<br>TCA 2.16% TRR 0.054 ppm<br>HA5 0.48% TRR 0.012 ppm<br>HA6 0.57% TRR 0.014 ppm<br>HLA6 0.25% TRR 0.006 ppm<br>HLMC4 0.55% TRR 0.014 ppm<br>Other unknowns 1.86% TRR 0.047 ppm<br>(ea $\leq$ 0.4% TRR) |

Table 5 (continued).

| Fraction                         | % TRR | ppm    | Characterization/Identification <sup>a</sup>  |
|----------------------------------|-------|--------|---|
| Aqueous*                         | 38.9  | 0.973  | <p><u>HPLC analysis resolved:</u></p> <p>Prohexadione 0.81% TRR 0.020 ppm</p> <p>Dioxopropyl prohexadione 6.33% TRR 0.158 ppm</p> <p>TCA 8.45% TRR 0.212 ppm</p> <p>HA5 1.42% TRR 0.036 ppm</p> <p>HA6 0.73% TRR 0.018 ppm</p> <p>HLA1 2.18% TRR 0.055 ppm</p> <p>HLA2 4.59% TRR 0.115 ppm</p> <p>HLA6 4.45% TRR 0.111 ppm</p> <p>Other unknowns 10.10% TRR 0.253 ppm<br/>(ea &lt;1.5% TRR)</p> <p><u>Additional characterization of selected fractions resolved:</u></p> <p>Polar metabolites &lt;3000 MW 2.56% TRR 0.064 ppm</p> <p>Base-labile conjugates 13.50% TRR 0.338 ppm</p> |
| Precipitate*                     | 12.7  | 0.318  | N/A.  |
| Nonextractable                   | N/R   | N/R    | Extracted with hexane.  |
| Hexane (lipids)*                 | 0.01  | <0.001 | N/A.  |
| Nonextractable                   | 37.7  | 0.943  | Subjected to extraction with sodium acetate buffer followed by: (i) carbohydrate and protein enzyme hydrolysis and lignin characterization via dioxane:2 N HCl; and (ii) mild base hydrolysis/molecular weight filtration.  |
| (ii) Buffer-soluble              | 6.41  | 0.160  | Characterized as cellular components.   |
| Nonextractable                   | 31.3  | 0.783  | Subjected to enzyme hydrolysis with hemicellulase, amylase, cellulysin, amyloglucosidase, and pectinase.  |
| Carbohydrate enzyme hydrolysate* | 4.73  | 0.118  | <p>Based on comparison of results with inactivated enzymes, 1.41% TRR (0.035 ppm) characterized as carbohydrates.</p> <p><u>HPLC analysis resolved:</u></p> <p>Prohexadione 1.70% TRR 0.042 ppm</p> <p>TCA 1.00% TRR 0.025 ppm</p> <p>Plus one additional unknown at 2.04% TRR (0.051 ppm).</p>   |
| Nonextractable                   | 26.6  | 0.665  | Subjected to enzyme hydrolysis with pronase.  |
| Protein hydrolysate*             | 3.06  | 0.077  | <p>Based on comparison of results with inactivated enzymes, 1.53% TRR (0.038 ppm) characterized as protein.</p> <p><u>HPLC analysis resolved:</u></p> <p>Prohexadione 1.41% TRR 0.035 ppm</p> <p>Plus two additional unknowns at 1.26% TRR (0.032 ppm) and 0.39% TRR (0.010 ppm).</p>   |
| Nonextractable                   | 23.5  | 0.589  | Extracted with dioxane:2 N HCl to characterize lignin.  |
| Dioxane:HCl (lignin)             | 13.6  | 0.345  | Concentrated to dryness, redissolved in water, and centrifuged.   |
| Aq-soluble*                      | 11.2  | 0.280  | <p><u>HPLC analysis resolved:</u></p> <p>Prohexadione 3.24% TRR 0.081 ppm</p> <p>TCA 3.28% TRR 0.082 ppm</p> <p>Plus one additional unknown at 3.68% TRR (0.092 ppm).</p>   |

Table 5 (continued).

| Fraction                    | % TRR | ppm   | Characterization/Identification <sup>a</sup>   |
|-----------------------------|-------|-------|--|
| Lignin*                     | 2.61  | 0.065 | Dissolved in dioxane:2 N HCl to produce dioxane extractable (7.10% TRR, 0.177 ppm) and dioxane insoluble fractions 0.560% TRR, 0.014 ppm).   |
| Nonextractable              | 6.13  | 0.153 | N/A.   |
| (ii) Buffer-soluble         | 6.85  | 0.171 | Filtered through 3000 MW filter.   |
| <3000 MW*                   | 6.12  | 0.153 | HPLC analysis resolved:<br>Dioxopropyl prohexadione 1.15% TRR 0.029 ppm<br>TCA 1.03% TRR 0.026 ppm<br>HA5 0.55% TRR 0.014 ppm<br>HA6 0.54% TRR 0.014 ppm<br>Other unknowns 2.85% TRR 0.071 ppm<br>(ea $\leq$ 1.26% TRR)  |
| >3000 MW*                   | 0.2   | 0.006 | N/A.   |
| Nonextractable              | 30.8  | 0.771 | Subjected to mild base hydrolysis with 1 N NaOH  |
| Base hydrolysate            | 11.7  | 0.294 | HPLC analysis resolved:<br>Prohexadione 2.26% TRR 0.057 ppm<br>Despropionyl prohexadione 2.54% TRR 0.064 ppm<br>TCA 1.89% TRR 0.048 ppm<br>Plus one additional unknown at 5.01% TRR (0.126 ppm).   |
| Nonextractable              | 18.3  | 0.459 | N/A.   |
| <b>Hay (TRR = 36.5 ppm)</b> |       |       |  |
| ACN:sulfuric acid           | 66.9  | 24.4  | Partitioned with DCM:ACN   |
| DCM*                        | 38.5  | 14.1  | HPLC analysis resolved:<br>Prohexadione 15.8% TRR 5.767 ppm<br>Dioxopropyl prohexadione 4.10% TRR 1.497 ppm<br>TCA 1.74% TRR 0.635 ppm<br>HA5 0.69% TRR 0.252 ppm<br>HA6 0.73% TRR 0.265 ppm<br>Other unknowns 15.5% TRR 5.68 ppm<br>(ea < 1.5% TRR)   |
| Aqueous*                    | 21.9  | 7.99  | HPLC analysis resolved:<br>Prohexadione 3.40% TRR 1.24 ppm<br>Dioxopropyl prohexadione 1.69% TRR 0.617 ppm<br>TCA 4.38% TRR 1.599 ppm<br>HA5 0.58% TRR 0.212 ppm<br>HA6 0.42% TRR 0.153 ppm<br>Other unknowns 11.4% TRR 4.17 ppm<br>(ea < 1% TRR)<br><br>Additional characterization of selected fractions resolved:<br>Polar metabolites <3000 MW 1.21% TRR 0.442 ppm<br>Base-labile conjugates 6.84% TRR 2.497 ppm |
| Nonextractable              | N/R   | N/R   | Extracted with hexane.   |
| Hexane (lipids)*            | 0.04  | 0.013 | HPLC analysis resolved:<br>Prohexadione 0.006% TRR 0.002 ppm<br>HH1 0.006% TRR 0.002 ppm<br>Lipids 0.024% TRR 0.009 ppm  |

Table 5 (continued).

| Fraction                         | % TRR | ppm   | Characterization/Identification <sup>a</sup>  |
|----------------------------------|-------|-------|---|
| Nonextractable                   | 41.7  | 15.2  | Subjected to extraction with sodium acetate buffer followed by: (I) carbohydrate and protein enzyme hydrolysis and lignin characterization via dioxane:2 N HCl; and (ii) mild base hydrolysis/molecular weight filtration.  |
| (I) Buffer-soluble               | 18.7  | 6.81  | Characterized as cellular components.   |
| Nonextractable                   | 23.0  | 8.39  | Subjected to enzyme hydrolysis with hemicellulase, amylase, cellulysin, amyloglucosidase, and pectinase.  |
| Carbohydrate enzyme hydrolysate* | 5.93  | 2.16  | Based on comparison of results with inactivated enzymes, 3.33% TRR (1.21 ppm) characterized as carbohydrates.<br><br><u>HPLC analysis resolved:</u><br>Prohexadione 1.55% TRR 0.564 ppm<br>Additional unknowns 4.38% TRR 1.596 ppm<br>(ea $\leq$ 1.43% TRR)             |
| Nonextractable                   | 17.1  | 6.23  | Subjected to enzyme hydrolysis with pronase.  |
| Protein hydrolysate*             | 2.51  | 0.916 | Based on comparison of results with inactivated enzymes, 0.66% TRR (0.2409 ppm) characterized as protein.<br><br><u>HPLC analysis resolved:</u><br>Prohexadione 0.69% TRR 0.250 ppm<br>Plus two additional unknowns at 1.55% TRR (0.566 ppm) and 0.27% TRR (0.100 ppm). |
| Nonextractable                   | 14.6  | 5.31  | Extracted with dioxane:2 N HCl to characterize lignin.  |
| Dioxane:HCl (lignin)             | 13.0  | 4.74  | Concentrated to dryness, redissolved in water, and centrifuged.   |
| Aq soluble                       | 9.31  | 3.39  | Subjected to mild base hydrolysis with 1 N NaOH followed by filtration on a 3000 MW filter.   |
| <3000 MW*                        | 10.3  | 3.73  | <u>HPLC analysis resolved:</u><br>Prohexadione 4.96% TRR 1.81 ppm<br>TCA 4.06% TRR 1.48 ppm<br>Plus one additional unknown at 1.23% TRR (0.448 ppm).  |
| >3000 MW                         | 0.07  | 0.027 | N/A.  |
| Aq insoluble (lignin)*           | 3.70  | 1.35  | Dissolved in dioxane:2 N HCl.   |
| Dioxane sol                      | 3.80  | 1.39  | Filtered on a 3000 MW filter.   |
| <3000 MW*                        | 4.15  | 1.52  | <u>HPLC analysis resolved:</u><br>Prohexadione 2.61% TRR 0.956 ppm<br>TCA 1.12% TRR 0.409 ppm<br>Plus one additional unknown at 1.15% TRR (0.421 ppm).  |
| >3000 MW                         | 0.38  | 0.137 | N/A.  |
| Dioxane insoluble                | 0.591 | 0.216 | N/A.  |
| Nonextractable                   | 3.11  | 1.13  | N/A.  |
| (ii) Buffer-soluble              | 17.1  | 6.24  | Filtered through 3000 MW filter.  |

Table 5 (continued).

| Fraction         | % TRR | ppm   | Characterization/Identification <sup>a</sup>   |
|------------------|-------|-------|--|
| <3000 MW*        | 15.7  | 5.73  | <u>HPLC analysis resolved:</u><br>Prohexadione 6.06% TRR 2.21 ppm<br>TCA 1.36% TRR 0.497 ppm<br>Other unknowns 8.28% TRR 3.02 ppm<br>(ea <1% TRR)  |
| >3000 MW*        | 1.51  | 0.550 | N/A.   |
| Nonextractable   | 24.6  | 8.97  | Subjected to mild base hydrolysis with 1 N NaOH  |
| Base hydrolysate | 13.1  | 4.76  | <u>HPLC analysis resolved:</u><br>Prohexadione 3.44% TRR 1.25 ppm<br>Despropionyl prohexadione 1.69% TRR 0.615 ppm<br>TCA 1.23% TRR 0.446 ppm<br>Other unknowns 6.74% TRR 2.45 ppm<br>(ea <1% TRR) |
| Nonextractable   | 9.69  | 3.53  | N/A.   |

<sup>a</sup> See Figure 1 for chemical names and structures of identified metabolites. Although structures were not proposed for HA5 and HA6, the petitioner concluded that these are oxidation products similar to dioxopropyl prohexadione. Analytical results for fractions **marked with an asterisk (\*)** are summarized in Table 6.

<sup>b</sup> N/R = Not reported.

Table 6. Summary of radioactive residues characterized/identified in peanut commodities harvested 22 days following treatment with [<sup>14</sup>C]prohexadione-calcium at a total application rate of ~1 lb ai/A (~2.7x the maximum proposed seasonal rate).

| Fraction                              | Nutmeats<br>(TRR = 4.15 ppm) |             | Hulls<br>(TRR = 2.50 ppm) |             | Hay<br>(TRR = 36.5 ppm) |              |
|---------------------------------------|------------------------------|-------------|---------------------------|-------------|-------------------------|--------------|
|                                       | % TRR                        | ppm         | % TRR                     | ppm         | % TRR                   | ppm          |
| <b>Identified <sup>a</sup></b>        |                              |             |                           |             |                         |              |
| Prohexadione                          | 38.3                         | 1.58        | 9.66                      | 0.24        | 35.08                   | 12.80        |
| Dioxopropyl prohexadione <sup>b</sup> | 2.08                         | 0.09        | 7.48                      | 0.19        | 5.79                    | 2.12         |
| TCA                                   | 3.06                         | 0.13        | 15.92                     | 0.40        | 12.66                   | 4.62         |
| <b>Total Identified</b>               | <b>43.44</b>                 | <b>1.80</b> | <b>33.06</b>              | <b>0.83</b> | <b>53.53</b>            | <b>19.54</b> |
| <b>Characterized <sup>c</sup></b>     |                              |             |                           |             |                         |              |
| HA5                                   | 5.63                         | 0.23        | 2.45                      | 0.06        | 1.27                    | 0.46         |
| HA6                                   | 6.01                         | 0.25        | 1.84                      | 0.05        | 1.15                    | 0.42         |
| NMC2                                  | 1.37                         | 0.057       | --                        | --          | --                      | --           |
| NMC3                                  | 1.00                         | 0.042       | --                        | --          | --                      | --           |
| NMC4                                  | 3.88                         | 0.161       | --                        | --          | --                      | --           |
| NA1                                   | 2.10                         | 0.087       | --                        | --          | --                      | --           |
| HLMC4                                 | --                           | --          | 0.55                      | 0.01        | --                      | --           |
| HLA1                                  | --                           | --          | 2.18                      | 0.06        | --                      | --           |
| HLA2                                  | --                           | --          | 4.59                      | 0.12        | --                      | --           |
| HLA6                                  | --                           | --          | 4.70                      | 0.12        | --                      | --           |
| HH1                                   | --                           | --          | --                        | --          | 0.01                    | <0.01        |
| Lipids                                | 3.01                         | 0.13        | 0.01                      | <0.001      | 0.02                    | 0.01         |
| Lignin                                | --                           | --          | 2.61                      | 0.06        | 3.70                    | 1.35         |
| Polar (>3000 MW)                      | 3.93                         | 0.16        | 0.20                      | 0.01        | 1.51                    | 0.550        |
| <b>Total identified/characterized</b> | <b>70.37</b>                 | <b>2.92</b> | <b>52.19</b>              | <b>1.32</b> | <b>61.19</b>            | <b>22.33</b> |
| Nonextractable                        | 1.10                         | 0.05        | 18.3                      | 0.46        | 9.69                    | 3.53         |

<sup>a</sup> See Figure 1 for chemical names and structures of identified metabolites. Although structures were not proposed for HA5 and HA6, the petitioner concluded that these are oxidation products similar to dioxopropyl prohexadione.

<sup>b</sup> Despropionyl prohexadione was only identified in the base hydrolysate of nonextractable residues following buffer extraction at 2.54% TRR (hulls) and 1.69% TRR (hay).

<sup>c</sup> The petitioner conducted additional procedures on nonextractable residues following solvent extraction which characterized residues as base-labile conjugates, sugars (osazones), carbohydrates, proteins, and polar metabolites <3000 MW. These data are presented in Table 5.

### Storage stability

Samples from the peanut metabolism study were stored frozen 349-537 days (~12-18 months) for hay and 370 days (~12 months) for nutmeat and hulls from harvest to extraction; actual analysis dates were not provided. To demonstrate the stability of prohexadione and metabolites in peanuts over the duration of the metabolism study, the petitioner conducted a storage stability study with a representative sample of nutmeats. A subsample of nutmeats was extracted and partitioned according to the procedures described above following frozen storage for 30 months. HPLC metabolite profiles for aqueous extracts and DCM fractions were compared for analyses conducted 12 months after sample collection and 30 months after collection. The data indicated that neither the extractability of radioactive residues nor the metabolite profile changed significantly over the study interval. These data are adequate to support the subject peanut metabolism study.

### Study summary

The peanut metabolism study is acceptable. Total radioactive residues were 4.15 ppm in peanut nutmeats, 2.50 ppm in hulls, and 36.5 ppm in hay collected 22 days following an over-the-top postemergence spray of [<sup>14</sup>C]prohexadione-calcium labeled at the C-3 and C-5 positions of the cyclohexenone ring at ~1.0 lb ai/A (~2.7x the maximum proposed seasonal rate of 0.375 lb ai/A).

Over 70% (nutmeats), 52% (hulls), and 61% (hay) of the TRR were characterized and identified. Prohexadione-calcium was rapidly metabolized to prohexadione (free acid). Prohexadione was the major residue identified in nutmeats (38.3% TRR, 1.58 ppm), hulls (9.66% TRR, 0.24 ppm), and hay (35.08% TRR, 12.80 ppm). The dioxopropyl prohexadione metabolite was identified in hulls (7.48% TRR, 0.19 ppm), nutmeats (2.08% TRR, 0.09 ppm) and hay (5.79% TRR, 2.12 ppm). The following additional metabolites were identified: despropionyl prohexadione in hulls (2.54% TRR, 0.064 ppm) and hay (1.69% TRR, 0.615 ppm); and TCA in nutmeats (3.06% TRR, 0.13 ppm), hulls (15.92% TRR, 0.40 ppm), and hay (12.66% TRR, 4.62 ppm).

Two unknowns, HA5 and HA6, characterized as oxidation products similar to dioxopropyl prohexadione accounted, respectively, for 5.63% and 6.01% TRR in nutmeats, 2.45% and 1.84% TRR in hulls, and 1.27% and 1.15% TRR in hay. In addition, residues characterized as lipids accounted for 3.01% TRR in nutmeats; residues characterized as lignin accounted for 2.61% and 3.70% TRR, respectively, in hulls and hay. The nonextractable residues in nutmeats, hulls, and hay were characterized as base-labile conjugates of prohexadione and its metabolites, sugars (osazones), carbohydrates, proteins, and polar metabolites <3000 MW.



### Proposed metabolic pathway for plants

Based on the results of the apple and peanut metabolism studies, the petitioner proposed that prohexadione-calcium undergoes extensive metabolism in plants. Prohexadione-calcium is extensively metabolized by a complex pathway, with numerous metabolites formed in small quantities and which are eventually assimilated into the carbon pool. Prohexadione-calcium is initially converted to prohexadione (free acid), which is further metabolized via successive  $\alpha$ -oxidation to the intermediate dioxopropyl prohexadione and to metabolites 27F2-A, despropionyl prohexadione, BX 112-M10, and to the natural products, tricarballic acid, and citric acid. Prohexadione is also oxidized via  $\beta$ -oxidation to BX 112-I5 and metabolite 27F1-A, and via ring oxidation to metabolite 27F2-B.

### Plant metabolism conclusions

The qualitative nature of the residue of prohexadione-calcium in plants is adequately understood for the purpose of this petition. The metabolism of prohexadione-calcium in apples and peanuts is similar. RAB1 will defer to the HED MARC on the toxicological significance of the prohexadione-calcium metabolites. A decision concerning which residues to regulate will then follow. A tolerance based on the parent only may not be appropriate; in such an instance a revised Section F, additional field studies, analytical methodology, and storage stability data may be needed.

### OPPTS GLN 860.1300: Nature of the Residue in Animals

#### Ruminants

BASF submitted data (citation listed below) pertaining to the metabolism of [ $^{14}\text{C}$ ]prohexadione-calcium in lactating goats. The biological and analytical phases of the study were conducted by Battelle Columbus Laboratories (Columbus, OH).

44457801 Chang, J.; et al. (1997) Metabolism of (Carbon 14)-BAS 125 W (Prohexadione-Calcium) in Lactating Goat. Lab Study Number SC940044. Unpublished study prepared by Battelle. 250 p.

The radioactive test substance was prepared by isotopic dilution of [ $^{14}\text{C}$ ]prohexadione-calcium (specific activity 61.1  $\mu\text{Ci}/\text{mg}$ , radiochemical purity >98%) with [ $^{13}\text{C}$ ]prohexadione-calcium and unlabeled prohexadione-calcium. Both [ $^{14}\text{C}$ ] and [ $^{13}\text{C}$ ]prohexadione-calcium were labeled at the C-3 and C-5 positions of the cyclohexenone ring. The test substance was dispensed into gelatin capsules to yield a formulated test substance with a final specific activity of 13.2  $\mu\text{Ci}/\text{mg}$  and radiochemical purity of 97%. The

capsules were administered orally by balling gun once per day for four consecutive days. A single goat received 206.3 mg of prohexadione-calcium per day. The dose rate was equivalent to 105 ppm of [<sup>14</sup>C]prohexadione-calcium per day based on the average daily food consumption (1971 g/day) during the dosing period. The daily dose rate was equivalent to 52x and 31x the maximum theoretical dietary burden for dairy and beef cattle, respectively; see "OPPTS GLN 860.1480: Meat, Milk, Poultry, Eggs" for calculation of dietary burden. A single goat was not treated and served as the control goat. During the study, the animals were fed commercial goat chow, alfalfa cubes, and hay, and water was provided *ad libitum*. The petitioner provided sufficient descriptions of preparation of dose capsules and animal husbandry practices as well as data concerning daily feed intake, body weights, and milk production.

Milk was collected twice daily (in the morning and afternoon). The afternoon milk sample was refrigerated (4 C) until combined with the following morning (prior to dosing) sample. The treated goat was sacrificed 20 hours after the final dose, and the control goat was sacrificed the following day. The following samples were collected: liver, kidneys, fat (including perirenal, omental, and mediastinal), and muscle. Tissue samples were refrigerated until cubed and random subsamples taken; subsampling was completed on the day of necropsy. All milk and tissue subsamples were frozen (~-20 C) until analysis.

#### Total radioactive residues (TRR)

Initial TRR determinations were made using small subsamples of milk and tissues. A second TRR determination was made using larger subsamples of tissues to be extracted for residue isolation and characterization; these values were used for all subsequent calculations. Triplicate aliquots of milk were analyzed directly by LSC. Subsamples of liver, kidney, fat, and muscle were homogenized under liquid N<sub>2</sub>, and triplicate aliquots were subjected to combustion/LSC. The TRR in goat milk and tissues are presented in Table 7. The LODs for TRR determinations ranged from 0.002 to 0.01 ppm depending on the goat matrix.

Table 7. Total radioactive residues in samples of milk and edible tissues from goats administered with [<sup>14</sup>C]prohexadione-calcium at 105 ppm for 4 consecutive days.

| Matrix | TRR, ppm [ <sup>14</sup> C]prohexadione-calcium equivalents |             |
|--------|---|-------------|
|        | Small Sample  | Bulk Sample |
| Milk   |   |             |
| Day 1  | 0.0604  | --          |
| Day 2  | 0.0799  | --          |
| Day 3  | 0.0816  | --          |
| Day 4  | 0.0884  | 0.082       |
| Kidney | 3.16  | 3.09        |
| Liver  | 0.432   | 0.427       |
| Muscle | 0.061   | 0.069       |
| Fat    | 0.054   | 0.048       |

Samples of urine, feces, and cage washings were collected daily, and were analyzed for total radioactivity. The study submission reported that 91.9% of the administered dose was recovered from the excreta: 72.8% in urine, 15.8% in feces, and 3.29% from cage washings. The petitioner submitted data indicating that 4.92% of the administered dose was also recovered from nonedible specimens (GI tract contents, rumen contents, blood, and bile), 0.24% from milk and tissues, and 0.09% from expired air (volatiles collected following the last dose). Approximately 97% of the administered dose was recovered.

#### Extraction and hydrolysis of residues

Day 4 milk and all tissue samples were subjected to extraction and/or hydrolysis procedures for residue characterization and identification. The petitioner provided adequate descriptions of the fractionation schemes used for the analysis of prohexadione-calcium residues in goat milk and tissues, and provided extraction procedures for each matrix. During the extraction and fractionation procedures, aliquots of extracts, hydrolysates, and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. Fractions containing significant residues were concentrated and reserved for chromatographic analysis. The general extraction and fractionation procedures are summarized below.

*Milk, fat, and muscle:* Radioactive residues in milk, fat, and muscle were extracted (3x) with ACN:1.5 M sulfuric acid (9:1, v:v) and centrifuged. The acidified ACN extracts were combined, concentrated by rotary evaporation, and partitioned (3x) with methylene chloride:ACN (3:1, v:v). The methylene chloride and aqueous (milk only) phases were reserved for TLC or HPLC analysis.

The postextraction solids from the ACN extractions were extracted (2x) with petroleum ether:diethylether (87:13, v:v).

*Kidney and liver:* Radioactive residues in liver and kidney were extracted as described for milk, fat, and muscle. The nonextractable residues remaining following petroleum ether extractions were dried and extracted (4x) with Tris buffer (pH 7.5) using sonication. The extracts were centrifuged, combined, and concentrated. The concentrated extract was filtered through a 3000 molecular weight cut-off membrane filter. The <3000 MW fraction was partitioned with methylene chloride. The nonextractable residues following Tris buffer extraction were digested twice with pronase enzyme in Tris buffer at 40 C for 24 hours. The enzyme hydrolysate was adjusted to pH 2 and partitioned with methylene chloride.

The petitioner validated the extraction procedures described above by fortifying subsamples of kidney and fat with [<sup>14</sup>C]prohexadione-calcium. [<sup>14</sup>C]Prohexadione was found to be adequately recovered using the extraction, partition, concentration, and HPLC analysis; recoveries were 96.9% and 101% for prohexadione in kidney and fat, respectively.

The distribution of <sup>14</sup>C-activity in the extracts and hydrolysates of goat milk and tissues is presented in Table 8.

#### Characterization and identification of residues

The organosoluble and other extracts described above were analyzed by several chromatographic techniques. Initial identification of prohexadione-calcium and its metabolites was achieved by HPLC and/or TLC co-chromatography with reference standards. For HPLC analyses, extracts were injected onto an HPLC system using UV detection with either a Hamilton PRP-1 or YMC ODS-Aq column and a gradient mobile phase of water with 0.1% acetic acid and ACN with 0.1% acetic acid. Fractions were collected and assayed by LSC. Residues were quantitated by peak areas, retention times, and co-chromatography with reference standards. The following reference standards were used for co-chromatography: prohexadione-calcium, prohexadione methyl ester, despropionyl prohexadione, and tricarballic acid.

Initial attempts to characterize the methylene chloride extract of milk using HPLC were unsuccessful. Therefore, residues were analyzed using two-dimensional TLC (2-D TLC) with co-chromatography with reference standards. 2-D TLC was also used to confirm residue identifications made using HPLC in the methylene chloride extracts of tissue samples. Analyses by 2-D TLC were performed using silica gel plates and isopropyl ether:formic acid:water (90:7:3, v:v:v). One-dimensional TLC was used to analyze buffer extracts and enzyme hydrolysates using the same solvent system as for 2-D TLC.

Radioimaging using an Ambis radioanalysis system was used to quantitate TLC isolated radioactive residues; nonradiolabeled standards were detected by UV (254 nm). Additional fractionation procedures were conducted on selected extracts, and these procedures are described below.

To further identify the radioactivity in the petroleum ether extract of milk, the extract was saponified with 2 N potassium hydroxide in methyl alcohol:water (50:50, v:v) at 100 C for 4 hours. The saponified solution was cooled and extracted (3x) with hexane to remove neutral lipids. No radioactivity was observed in the hexane fraction; the aqueous fraction was acidified to pH 1 and extracted (4x) with methylene chloride. The methylene chloride extract was concentrated and analyzed by HPLC/UV using a UHS C18 column and a gradient mobile phase of water with 0.1% acetic acid and ACN with 0.1% acetic acid and tetrahydrofuran. HPLC resolved the radioactivity in the region where fatty acids eluted.

To determine if some radioactivity in milk had been incorporated into sugars, subsamples of the aqueous fraction was subjected to osazone derivatization. The aqueous fraction was heated at 30 C for 45 minutes with sulfuric acid, diluted with water, and calcium carbonate was added until the pH was 7. Calcium sulfate was precipitated by centrifugation, and the precipitate was rinsed with water. The precipitate rinse and supernatant were combined, concentrated, and heated to 100 C with phenylhydrazine-HCl and sodium acetate for one hour. Crystals formed following refrigeration (~4 C) for 2 days. The crystals were dissolved in ethanol, re-precipitated by the addition of water, and then filtered. Three re-crystallizations yielded consistent radioactivity demonstrating that the radiolabel was incorporated into the sugar backbone.

Initial HPLC analysis of the aqueous fractions of kidney and liver resolved two despropionyl metabolites in kidney and one in liver. The first kidney metabolite was cochromatographed with despropionyl prohexadione following base hydrolysis. Base hydrolysis of the liver metabolite gave the same results as for kidney, thus confirming that it was also a despropionyl prohexadione precursor. The second kidney metabolite was isolated by extracting/partitioning a separate subsample and then clean-up by SPE to remove matrix interferences. Following matrix removal, the residue was subjected to a mild acid hydrolysis (pH adjusted to 1; heated for 1 hour at 60 C with 1 N HCl). The hydrolysate peak was subjected to semi-preparative HPLC and later co-chromatographed with despropionyl prohexadione using HPLC. The identity of the isolated metabolite was confirmed by methylation of the metabolite hydrolysate and comparison of GC/MS spectra with dimethyl despropionyl prohexadione standard.

The major residues in the aqueous fraction of liver were characterized as carboxylic acids. This determination was made following conduct of procedures including acid/base hydrolyses, semi-preparative HPLC and SPE chromatographic cleanup, HPLC co-chromatography with tricarballic acid (isolated from peanut hay) and carboxylic acids, methylation, and derivatization with phenyl isothiocyanate. The combined procedures and results indicated that the metabolite was not tricarballic acid, citric acid, fumaric acid, or succinic acid but exhibited chromatographic properties similar to low molecular weight carboxylic acids. Attempts to confirm this characterization using GC/MS and LC/MS failed due to high polarity and lack of material.

A summary of the characterized and identified  $^{14}\text{C}$ -residues in goat matrices is presented in Table 9.

Table 8. Distribution of total radioactive residues in milk and tissues from a goat dosed with [<sup>14</sup>C]prohexadione-calcium at 105 ppm in the diet for 4 consecutive days.

| Fraction                             | % TRR           | ppm   | Characterization/Identification <sup>a</sup>   |
|--------------------------------------|-----------------|-------|--|
| <b>Milk, Day 4 (TRR = 0.082 ppm)</b> |                 |       |  |
| ACN                                  | 61.3            | 0.050 | Concentrated and partitioned (3x) with methylene chloride:ACN.   |
| Methylene chloride                   | 30.2            | 0.025 | <u>2D-TLC analysis resolved:</u><br>Prohexadione 19.0% TRR 0.016 ppm<br>Unknown A 0.97% TRR <0.001 ppm<br>Unknown B 1.30% TRR 0.001 ppm<br>Unknown D 8.91% TRR 0.007 ppm   |
| Aqueous                              | 25.6            | 0.021 | <u>HPLC analysis resolved:</u><br>Sugars* 22.6% TRR 0.019 ppm<br>Unknown Rt 98.5 2.98% TRR 0.002 ppm<br>*Sugars were characterized by osazone derivatization using a separate milk subsample.  |
| Nonextractable                       | NR <sup>b</sup> | NR    | Extracted (2x) with petroleum ether:diethylether.  |
| Petroleum ether                      | 15.2            | 0.012 | Saponified with 2 N potassium hydroxide in methyl alcohol:water at 100 C for 4 hours. HPLC resolved the radioactivity in the region of fatty acids (lipids).   |
| Nonextractable                       | 17.4            | 0.014 | Not further analyzed (N/A).  |
| <b>Kidney (TRR = 3.09 ppm)</b>       |                 |       |  |
| ACN                                  | 89.8            | 2.78  | Concentrated and partitioned (3x) with methylene chloride:ACN.   |
| Methylene chloride                   | 41.9            | 1.30  | <u>HPLC analysis resolved:</u><br>Prohexadione 37.1% TRR 1.15 ppm<br>Unknown KMC1 2.10% TRR 0.065 ppm<br>Unknown KMC2 0.39% TRR 0.012 ppm<br>Unknown KMC3 0.67% TRR 0.021 ppm<br>Unknown KMC4 1.51% TRR 0.047 ppm<br>Unknown KMC5 0.16% TRR 0.005 ppm  |
| Aqueous                              | 46.8            | 1.45  | <u>HPLC analysis resolved:</u><br>Prohexadione 1.11% TRR 0.034 ppm<br>Despropionyl prohexadione 31.7% TRR 0.980 ppm<br>and precursor metabolites*<br>Unknown KA1 2.95% TRR 0.091 ppm<br>Unknown KA2 4.94% TRR 0.153 ppm<br>Unknown KA3 4.66% TRR 0.144 ppm<br>Unknown KA6 1.39% TRR 0.043 ppm<br>*Despropionyl precursor metabolites were identified by HPLC and GC/MS co-chromatography with despropionyl prohexadione following acid/base hydrolyses and/or methylation. |
| Nonextractable                       | NR              | NR    | Extracted (2x) with petroleum ether:diethylether.  |
| Petroleum ether                      | 0.12            | 0.004 | N/A.   |
| Nonextractable                       | 18.8            | 0.581 | Extracted (4x) with Tris buffer (pH 7.5)   |

Table 8 (continued).

| Fraction                       | % TRR | ppm   | Characterization/Identification *  |
|--------------------------------|-------|-------|--|
| Buffer extract                 | NR    | NR    | Molecular weight fractionation through a 3000 MW cut-off membrane filter.  |
| MW>3000                        | 2.66  | 0.082 | N/A.   |
| MW<3000                        | 5.26  | 0.163 | Partitioned with methylene chloride.   |
| Methylene Cl                   | 1.77  | 0.055 | <u>1-D TLC analysis resolved:</u><br>Prohexadione 1.42% TRR 0.044 ppm  |
| Aqueous                        | 2.84  | 0.088 | 1-D TLC: components were retained at the origin.   |
| Nonextractable                 | NR    | NR    | Digested with pronase enzyme.  |
| Hydrolysate                    | 8.69  | 0.268 | Partitioned with methylene chloride.   |
| Methylene Cl                   | 1.60  | 0.049 | N/A.   |
| Aqueous                        | 7.25  | 0.224 | <u>HPLC analysis resolved:</u><br>Prohexadione 1.26% TRR 0.039 ppm<br>Polar components each <3.00% TRR 0.008 ppm   |
| Nonextractable                 | 1.79  | 0.055 | N/A.   |
| <b>Liver (TRR = 0.427 ppm)</b> |       |       |  |
| ACN                            | 73.0  | 0.312 | Concentrated and partitioned (3x) with methylene chloride:ACN.   |
| Methylene chloride             | 31.8  | 0.136 | <u>HPLC analysis resolved:</u><br>Prohexadione 29.7% TRR 0.127 ppm<br>Unknown LMC1 2.11% TRR 0.009 ppm   |
| Aqueous                        | 46.3  | 0.198 | <u>HPLC analysis resolved:</u><br>Despropionyl prohexadione and precursor metabolites* 10.5% TRR 0.045 ppm<br>Carboxylic acids** 20.2% TRR 0.086 ppm<br>Unknown LA1 5.51% TRR 0.024 ppm<br>Unknown LA3 5.13% TRR 0.022 ppm<br>Unknown LA4 5.03% TRR 0.022 ppm<br><br>*Despropionyl precursor metabolites were identified by HPLC and GC/MS co-chromatography with despropionyl prohexadione following acid/base hydrolyses and/or methylation.<br>**Carboxylic acids were characterized based on chromatographic properties using two different HPLC systems (reverse phase and ion modulated partition chromatography). |
| Nonextractable                 | NR    | NR    | Extracted (2x) with petroleum ether:diethylether.  |
| Petroleum ether                | 0.76  | 0.003 | N/A.   |
| Nonextractable                 | 23.8  | 0.102 | Extracted (4x) with Tris buffer (pH 7.5)   |
| Buffer extract                 | NR    | NR    | Molecular weight fractionation through a 3000 MW cut-off membrane filter.  |
| MW>3000                        | 3.01  | 0.013 | N/A.   |
| MW<3000                        | 6.60  | 0.028 | Partitioned with methylene chloride.   |
| Methylene Cl                   | 1.27  | 0.005 | <u>1-D TLC analysis resolved:</u><br>Prohexadione 0.9% TRR 0.004 ppm   |



Table 8 (continued).

| Fraction                        | % TRR | ppm    | Characterization/Identification <sup>a</sup>                      |
|---------------------------------|-------|--------|---|
| Aqueous                         | 6.18  | 0.027  | 1-D TLC: components were retained at the origin.                  |
| Nonextractable                  | NR    | NR     | Digested with pronase enzyme.                                     |
| Hydrolysate                     | 15.4  | 0.066  | Partitioned with methylene chloride.                              |
| Methylene Cl                    | 2.17  | 0.009  | N/A.  |
| Aqueous                         | 11.7  | 0.050  | HPLC analysis resolved:<br>Two unknowns each present at <0.05 ppm |
| Nonextractable                  | 2.21  | 0.009  | N/A.  |
| <b>Muscle (TRR = 0.069 ppm)</b> |       |        |   |
| ACN                             | 80.9  | 0.056  | Concentrated and partitioned (3x) with methylene chloride:ACN.    |
| Methylene chloride              | 73.5  | 0.051  | HPLC analysis resolved:<br>Prohexadione 73.5% TRR 0.051 ppm       |
| Aqueous                         | 10.4  | 0.007  | N/A.  |
| Nonextractable                  | NR    | NR     | Extracted (2x) with petroleum ether:diethylether.                 |
| Petroleum ether                 | 0.337 | <0.001 | N/A.  |
| Nonextractable                  | 17.2  | 0.012  | N/A.  |
| <b>Fat (TRR = 0.048 ppm)</b>    |       |        |   |
| ACN                             | 102   | 0.049  | Concentrated and partitioned (3x) with methylene chloride:ACN.    |
| Methylene chloride              | 89.3  | 0.043  | HPLC analysis resolved:<br>Prohexadione 89.3% TRR 0.043 ppm       |
| Aqueous                         | 9.05  | 0.004  | N/A.  |
| Nonextractable                  | NR    | NR     | Extracted (2x) with petroleum ether:diethylether.                 |
| Petroleum ether                 | 4.45  | 0.002  | N/A.  |
| Nonextractable                  | 11.1  | 0.005  | N/A.  |

<sup>a</sup> Initial identification of prohexadione by HPLC was confirmed using 2-D TLC.

<sup>b</sup> NR = Not reported.

Table 9. Summary of radioactive residues characterized/identified in milk and tissues of a lactating goat orally dosed with [<sup>14</sup>C]prohexadione-calcium at 105 ppm for 4 consecutive days.

| Fraction   | Milk, Day 4<br>(TRR = 0.082 ppm) |              | Kidney<br>(TRR = 3.09 ppm) |              | Liver<br>(TRR = 0.427 ppm) |              | Muscle<br>(TRR = 0.069 ppm) |              | Fat<br>(TRR = 0.048 ppm) |              |
|--|----------------------------------|--------------|----------------------------|--------------|----------------------------|--------------|-----------------------------|--------------|--------------------------|--------------|
|  | % TRR                            | ppm          | % TRR                      | ppm          | % TRR                      | ppm          | % TRR                       | ppm          | % TRR                    | ppm          |
| <b>Identified <sup>a</sup></b>                         |                                  |              |                            |              |                            |              |                             |              |                          |              |
| Prohexadione   | 19.0                             | 0.016        | 40.9                       | 1.267        | 30.6                       | 0.131        | 73.5                        | 0.051        | 89.3                     | 0.043        |
| Despropionyl prohexadione<br>and precursor metabolites | --                               | --           | 31.7                       | 0.980        | 10.5                       | 0.045        | --                          | --           | --                       | --           |
| <b>Total identified</b>                                | <b>19.0</b>                      | <b>0.016</b> | <b>72.6</b>                | <b>2.247</b> | <b>41.1</b>                | <b>0.176</b> | <b>73.5</b>                 | <b>0.051</b> | <b>89.3</b>              | <b>0.043</b> |
| <b>Characterized</b>                                   |                                  |              |                            |              |                            |              |                             |              |                          |              |
| Low molecular weight<br>carboxylic acids               | --                               | --           | --                         | --           | 20.2                       | 0.086        | --                          | --           | --                       | --           |
| Sugars   | 22.6                             | 0.019        | --                         | --           | --                         | --           | --                          | --           | --                       | --           |
| Lipids   | 15.2                             | 0.012        | --                         | --           | --                         | --           | --                          | --           | --                       | --           |
| Unknown peaks  | 14.2                             | <0.011       | 18.8                       | 0.581        | 17.8                       | 0.077        | --                          | --           | --                       | --           |
| Aqueous  | --                               | --           | --                         | --           | --                         | --           | 10.4                        | 0.007        | 9.1                      | 0.004        |
| Petroleum ether extract                                | --                               | --           | 0.1                        | 0.004        | 0.8                        | 0.003        | 0.3                         | <0.001       | 4.5                      | 0.002        |
| >3000 MW   | --                               | --           | 2.7                        | 0.082        | 3.0                        | 0.013        | --                          | --           | --                       | --           |
| Aqueous (<3000 MW)                                     | --                               | --           | 2.8                        | 0.088        | 6.2                        | 0.027        | --                          | --           | --                       | --           |
| Methylene chloride<br>(pronase hydrolysate)            | --                               | --           | 1.6                        | 0.049        | 2.2                        | 0.009        | --                          | --           | --                       | --           |
| <b>Total identified and<br/>characterized</b>          | <b>71.0</b>                      | <b>0.058</b> | <b>98.6</b>                | <b>3.051</b> | <b>91.3</b>                | <b>0.391</b> | <b>84.2</b>                 | <b>0.059</b> | <b>102.9</b>             | <b>0.049</b> |
| Nonextractable   | 17.4                             | 0.014        | 1.8                        | 0.055        | 2.2                        | 0.009        | 17.2                        | 0.012        | 11.1                     | 0.005        |

<sup>a</sup> See Figure 1 (Attachment II) for chemical names and structures of identified metabolites.

### Storage stability

The petitioner submitted dates indicating that extraction of milk and tissue samples were conducted within ~4 months of sample collection; analysis dates were not provided but the petitioner states that quantitation of residues in milk and tissues was completed within 4 months of sample collection. To demonstrate the stability of prohexadione-calcium residues over the course of the study, the petitioner conducted a storage stability study using goat kidney which contained the highest radioactive residues. Subsamples of kidney were analyzed ~2 months and ~12 months after storage at -20 C. Prohexadione was relatively stable in goat kidney for up to 12 months. Degradation of the despropionyl prohexadione metabolites to despropionyl prohexadione and to polar metabolites was observed. Extraction flow charts, tabulated results, and chromatographic data for the storage stability samples were provided; these data are adequate to support the subject goat metabolism study.

### Study summary

The goat metabolism study is acceptable. Following oral administration of [<sup>14</sup>C]prohexadione-calcium to lactating goats for four consecutive days at 105 ppm, the TRR were <0.0604-0.0884 ppm in milk, 3.09-3.16 ppm in kidney, 0.427-0.432 ppm in liver, 0.061-0.069 ppm in muscle, and 0.048-0.054 ppm in fat.

Approximately 71-103% of the TRR were characterized/identified in milk and tissues. Prohexadione-calcium was converted to prohexadione as the free acid. Prohexadione was the major residue identified in milk (19.0% TRR, 0.016 ppm), kidney (40.9% TRR, 1.267 ppm), liver (30.6% TRR, 0.131 ppm), muscle (73.5% TRR, 0.051 ppm), and fat (89.3% TRR, 0.043 ppm). Metabolites related to, or a precursor of, despropionyl prohexadione were identified in kidney (31.7% TRR, 0.980 ppm) and liver (10.5% TRR, 0.045 ppm). Polar and low molecular weight metabolites were characterized as carboxylic acids in liver (20.2% TRR, 0.086 ppm). Milk contained radioactivity incorporated into sugars (22.6% TRR, 0.019 ppm) and lipids (15.2% TRR, 0.012 ppm), suggesting metabolism of prohexadione to the carbon pool.

### Poultry

BASF submitted a study (citation listed below) investigating the metabolism of [<sup>14</sup>C]prohexadione-calcium in laying hens. The biological and analytical phases of the study were conducted by Battelle Columbus Laboratories (Columbus, OH).

44457794 Steginsky, C.; Cristy, T.; Trigg, N. et al. (1997) Metabolism of (carbon 14)-BAS 125 W (Prohexadione Calcium) in

Egg-Laying White Leghorn Hens: Final Report: Lab Project Number: SC930241: 93149: M9513. Unpublished study prepared by Battelle.

The radioactive test substance was prepared by isotopic dilution of [ $^{14}\text{C}$ ]prohexadione-calcium (specific activity 61.1  $\mu\text{Ci}/\text{mg}$ , radiochemical purity >98%) with [ $^{13}\text{C}$ ]prohexadione-calcium and unlabeled prohexadione-calcium. [ $^{14}\text{C}$ ] and [ $^{13}\text{C}$ ]Prohexadione-calcium were labeled with  $^{14}\text{C}$  and  $^{13}\text{C}$  at the C-3 and C-5 positions of the cyclohexenone ring. The test substance was dissolved in acetonitrile and dispensed into gelatin capsules to yield a formulated test substance with a final specific activity of 30.9  $\mu\text{Ci}/\text{mg}$  and radiochemical purity of 96%. The capsules were administered orally once per day for five consecutive days. One group of five hens received 1.20 mg of prohexadione-calcium per day, and another group of ten hens received 4.80 mg of prohexadione-calcium per day. The dose rates were equivalent to 8.43 ppm (low dose) and 33.4 ppm (high dose) of [ $^{14}\text{C}$ ]prohexadione-calcium per day, respectively, based on average daily food consumption of 120 g/day. The daily doses were equivalent to ~34x (low dose) and 134x (high dose) the maximum theoretical dietary burden for poultry. The dietary burden was calculated based on the 25% contribution of peanut meal in the diet and a proposed tolerance of 1 ppm for the RAC. A group of five hens was not treated and served as a control group. During the study, the hens were fed commercial laying mash, and water was provided *ad libitum*. The petitioner provided sufficient descriptions of preparation of dose capsules and animal husbandry practices as well as data concerning daily feed intake, body weights, and egg production.

Eggs were collected twice daily (in the morning and afternoon). Morning eggs were pooled with eggs collected from the previous afternoon, and after pooling, the whites and yolks were separated. The hens were sacrificed 20-21 hours after the final dose, and the following samples were collected: liver, kidneys, gizzard, breast and thigh muscle, fat, and skin with fat. Tissues were combined within each group. Whole eggs found in the oviduct were combined with the last egg collection. Blood and the GI tract and contents were also collected; however, analytical data from the analyses of these samples are not presented in this document. Eggs and tissues were stored refrigerated until they were processed (minced, stirred, or homogenized) for sample preparation. All egg and tissue samples were then stored frozen ( $\sim -20^\circ\text{C}$ ) until analysis.

#### Total radioactive residues (TRR)

Triplicate aliquots of egg whites and yolks, kidney, liver, fat, skin, and muscle were homogenized and analyzed by combustion/LSC. The results of TRR determinations are presented in Table 10. The LOD for TRR determinations was reported as 0.002-0.01 ppm.

Table 10. Total radioactive residues in samples of eggs and edible tissues from hens following administration of [<sup>14</sup>C]prohexadione at 8.43 (low dose) or 33.4 (high dose) ppm for 5 consecutive days.

| Matrix        | TRR, ppm [ <sup>14</sup> C]prohexadione-calcium equivalents |                     |                    |
|---------------|---|---------------------|--------------------|
|               | Low Dose  | High Dose - Average | High Dose - Pooled |
| Egg, yolks    |   |                     |                    |
| Day 1         | <0.008  | <0.008              | --                 |
| Day 2         | <0.008  | <0.008              | --                 |
| Day 3         | <0.008  | 0.009               | --                 |
| Day 4         | <0.008  | 0.015               | --                 |
| Day 5         | <0.008  | 0.019               | 0.022              |
| Egg, whites   |   |                     |                    |
| Day 1         | <0.005  | <0.005              | --                 |
| Day 2         | <0.005  | 0.011               | --                 |
| Day 3         | <0.005  | 0.013               | --                 |
| Day 4         | <0.005  | 0.015               | --                 |
| Day 5         | <0.005  | 0.015               | 0.014              |
| Gizzard       | 0.005   | 0.021               | 0.021              |
| Kidneys       | 0.0936  | 0.475               | 0.471              |
| Liver         | 0.007   | 0.029               | 0.030              |
| Muscle        |   |                     |                    |
| Breast        | <0.007  | <0.007              | --                 |
| Thigh         | <0.005  | 0.010               | 0.011              |
| Fat           | <0.01   | <0.01               | --                 |
| Skin with fat | 0.006   | 0.022               | --                 |

Samples of excreta collected once daily and the water cage rinse collected on the day of termination were also analyzed for TRR. The data indicated that 92.6-96.3% of the administered dose was recovered from the excreta and cage wash. The petitioner submitted data indicating that 92.7-96.6% of the total administered dose was recovered from eggs, tissues, blood, and excreta.

#### Extraction and hydrolysis of residues

Samples with TRR >0.01 ppm were subjected to extraction and/or hydrolysis procedures for residue characterization and identification; we note that no fractionation procedures were performed on skin with fat (TRR=0.022 ppm). The petitioner provided adequate descriptions of the fractionation schemes for each matrix. During the extraction and fractionation procedures, aliquots of extracts, hydrolysates, and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. Fractions containing significant residues were concentrated and

reserved for chromatographic analysis. The general extraction and fractionation procedures are summarized below.

*Gizzard and thigh muscle:* Radioactive residues in gizzard and thigh muscle were extracted (3x) with ACN:1.5 M sulfuric acid (9:1, v:v), centrifuged, and the acidified ACN extracts were combined. The postextraction solids from the ACN extractions were extracted (2x) with petroleum ether:diethylether (87:13, v:v), centrifuged, and combined.

*Egg whites, kidney, and liver:* Radioactive residues in egg whites and liver were extracted as described for gizzard and thigh muscle. The combined ACN extract was concentrated by rotary evaporation and partitioned (3x) with methylene chloride:ACN (3:1, v:v). The methylene chloride phases and aqueous phases (kidney only) were respectively combined and reserved for HPLC analysis.

The postextraction solids of kidney, following petroleum ether extraction, were subjected to buffer extraction, enzyme hydrolysis (Pronase), molecular weight filtration, and partitioning with methylene chloride. Subsamples of kidney's nonextractable residues were dried and extracted (3x) with 0.05 M Tris:0.01 M calcium chloride buffer (pH 7.5) using sonication. The extracts were centrifuged, combined, and concentrated. The concentrated extract was adjusted to pH 3 and partitioned with methylene chloride resulting in three layers: an aqueous layer; a semi-solid gelatinous layer believed to contain macromolecules; and an organic layer. The organic layer was reserved for 1-D TLC analysis. The nonextractable residues from the Tris buffer extraction were further characterized by enzymatic hydrolysis (digested with pronase enzyme in Tris buffer at 40 C for 18 hours).

*Egg yolks:* Radioactive residues in egg yolks were extracted as described for egg whites; however, the concentrated ACN extract was sequentially partitioned with methylene chloride:ACN (3:1, v:v) and methylene chloride. The methylene chloride fractions were combined and reserved for HPLC analysis.

The petitioner validated the extraction/concentration procedures using control liver samples fortified with an acidified solution of [ $^{14}\text{C}$ ]prohexadione-calcium. [ $^{14}\text{C}$ ]Prohexadione was found to be adequately recovered using the extraction, partition, concentration, and HPLC analysis procedures. Recoveries at various stages of the extraction procedure were 86.5-99.8% for prohexadione in liver.

The distribution of  $^{14}\text{C}$ -activity in the extracts and hydrolysates of hen eggs and tissues is presented in Table 11.

## Characterization and identification of residues

Identification of prohexadione-calcium and its metabolites was achieved by HPLC and/or TLC co-chromatography with reference standards. For HPLC analyses, extracts were injected onto an HPLC system using UV detection with either a Hamilton PRP-1 or YMC ODS-Aq column and a gradient mobile phase of water with 0.1% acetic acid and ACN with 0.1% acetic acid. Residues were quantitated by peak areas, retention times, and co-chromatography with reference standards. The following reference standards were used for co-chromatography: prohexadione-calcium, prohexadione methyl ester (BW 9054-M7), despropionyl prohexadione (BW 9054-5376), glutaric acid, and tricarballic acid (TCA).

The organic layer from the Tris buffer extraction of kidney was analyzed using 1-D TLC on silica gel plates developed with isopropyl ether:formic acid:water (90:7:3, v:v:v). Radioactive areas were located and quantified by Ambis radioanalysis system, visualized under UV light 254 nm, and identified by comparison to reference standards which have been co-chromatographed with the test solution. 2-D TLC was used to confirm the identities of residues in the methylene chloride extracts.

Prohexadione was identified as the major peak in the methylene chloride extracts of egg whites and yolks, kidney, and liver. Tricarballic acid was identified in the aqueous extract of kidney. A metabolite, designated as Metabolite 1, was resolved in egg whites and liver. To further characterize/identify Metabolite 1, aliquots of the egg white's methylene chloride extract were subjected to mild base (0.5 N NaOH at 40 C for 4 hours), strong acid (1 N HCl overnight at 65 C), strong base (1 N NaOH overnight at 65 C), and enzyme (incubated for 15 minutes with esterase in borate buffer) hydrolysis. HPLC of the hydrolysates indicated that Metabolite 1 was degraded by strong acid or base hydrolysis, but was resistant to mild base and enzyme (esterase) hydrolysis. Attempts to further characterize Metabolite 1 by methylating the extract of a pooled sample of the remaining high dose egg whites, and GC/MS and LC/MS analysis were unsuccessful. Because of the limited amount of material additional characterization of Metabolite 1 was not performed.

A summary of the characterized and identified <sup>14</sup>C-residues in hen matrices is presented in Table 12. Gizzard and thigh muscle are not included in the summary table because no residues were identified in these matrices.

Table 11. Distribution of total radioactive residues in egg and tissues from hens dosed with [ $^{14}\text{C}$ ]prohexadione-calcium at 33.4 ppm (high dose) in the diet for 5 consecutive days.

| Fraction                                  | % TRR           | ppm    | Characterization/Identification <sup>a</sup>   |
|---|-----------------|--------|--|
| <b>Egg yolk, Day 5 (TRR = 0.022 ppm)</b>  |                 |        |  |
| ACN                                       | 40.4            | 0.009  | Concentrated to aqueous; partitioned with methylene chloride:ACN and methylene chloride (3x).  |
| Methylene chloride                        | 28.6            | 0.006  | <u>HPLC analysis resolved:</u><br>Prohexadione 12.5% TRR 0.003 ppm<br>Metabolite 1 2.3% TRR 0.001 ppm<br>Unknown 4 Rt 3.0% TRR 0.001 ppm<br>Unknown 5 Rt 2.7% TRR 0.001 ppm<br>Unknown 6 Rt 3.6% TRR 0.001 ppm<br>Unknown 7.5 Rt 1.4% TRR <0.001 ppm<br>Unknown 24.5 Rt 3.1% TRR 0.001 ppm |
| Aqueous                                   | 12.7            | 0.003  | Not further analyzed (N/A).  |
| Nonextractable                            | NR <sup>b</sup> | NR     | Extracted (2x) with petroleum ether:diethylether.  |
| Petroleum ether                           | 12.1            | 0.003  | N/A.   |
| Nonextractable                            | 42.9            | 0.009  | N/A.   |
| <b>Egg white, Day 5 (TRR = 0.014 ppm)</b> |                 |        |  |
| ACN                                       | 101             | 0.014  | Concentrated to aqueous; partitioned (3x) with methylene chloride:ACN.   |
| Methylene chloride                        | 103             | 0.014  | <u>HPLC analysis resolved:</u><br>Prohexadione 27.3% TRR 0.004 ppm<br>Metabolite 1 71.5% TRR 0.010 ppm<br>Unknown 5.5 Rt 2.4% TRR <0.001 ppm<br>Unknown 25 Rt 2.7% TRR <0.001 ppm  |
| Aqueous                                   | 5.6             | 0.001  | N/A.   |
| Nonextractable                            | NR              | NR     | Extracted (2x) with petroleum ether:diethylether.  |
| Petroleum ether                           | 0.8             | <0.001 | N/A.   |
| Nonextractable                            | 11.0            | 0.002  | N/A.   |
| <b>Gizzard (TRR = 0.021 ppm)</b>          |                 |        |  |
| ACN                                       | 47.3            | 0.010  | N/A.   |
| Nonextractable                            | NR              | NR     | Extracted (2x) with petroleum ether:diethylether.  |
| Petroleum ether                           | 1.6             | <0.001 | N/A.   |
| Nonextractable                            | 63.4            | 0.013  | N/A.   |
| <b>Kidney (TRR = 0.471 ppm)</b>           |                 |        |  |
| ACN                                       | 35.5            | 1.67   | Concentrated to aqueous; partitioned (3x) with methylene chloride:ACN.   |
| Methylene chloride                        | 14.7            | 0.069  | <u>HPLC analysis resolved:</u><br>Prohexadione 11.9% TRR 0.056 ppm<br>Metabolite 1 1.6% TRR 0.007 ppm<br>Unknown 3 Rt 1.1% TRR 0.005 ppm<br>Unknown 27 Rt 0.1% TRR <0.001 ppm  |



Table 11 (continued).

| Fraction                               | % TRR | ppm    | Characterization/Identification <sup>a</sup>                           |
|--|-------|--------|--|
| Aqueous                                | 18.9  | 0.089  | <u>HPLC analysis resolved:</u>   |
|  |       |        | Prohexadione 1.3% TRR 0.006 ppm  |
|  |       |        | TCA 15.5% TRR 0.073 ppm  |
|  |       |        | Unknown 30.5 Rt 0.9% TRR 0.004 ppm                                     |
|  |       |        | Unknown 83 Rt 0.9% TRR 0.004 ppm                                       |
|  |       |        | Unknown 86.5 Rt 0.8% TRR 0.004 ppm                                     |
| Nonextractable                         | NR    | NR     | Extracted (2x) with petroleum ether:diethylether.                      |
| Petroleum ether                        | 0.3   | 0.001  | N/A.   |
| Nonextractable                         | 58.6  | 0.276  | Extracted (3x) with Tris buffer (pH 7.5)                               |
| Buffer extract                         | 33.2  | 0.156  | Acidified to pH 3; partitioned with methylene chloride.                |
| Semi-solid gel                         | 9.3   | 0.044  | N/A; believed to contain macromolecules.                               |
| Methylene chloride                     | 14.5  | 0.068  | <u>HPLC analysis resolved:</u>   |
|  |       |        | Prohexadione 14.5% TRR 0.068 ppm                                       |
| Aqueous                                | 6.6   | 0.031  | N/A.   |
| Nonextractable                         | NR    | NR     | Digested with Pronase enzyme.  |
| Pronase hydrolysate                    | 8.4   | 0.040  | N/A.   |
| Nonextractable                         | 22.0  | 0.104  | N/A.   |
| <b>Liver (TRR = 0.030 ppm)</b>         |       |        |  |
| ACN                                    | 52.5  | 0.016  | Concentrated to aqueous; partitioned (3x) with methylene chloride:ACN. |
| Methylene chloride                     | 34.7  | 0.011  | <u>HPLC analysis resolved:</u>   |
|  |       |        | Prohexadione 14.9% TRR 0.005 ppm                                       |
|  |       |        | Unknown 3.3 Rt 1.7% TRR 0.001 ppm                                      |
|  |       |        | Unknown 20 Rt 18.2% TRR 0.006 ppm                                      |
| Aqueous                                | 17.6  | 0.005  | N/A.   |
| Nonextractable                         | NR    | NR     | Extracted (2x) with petroleum ether:diethylether.                      |
| Petroleum ether                        | 2.3   | 0.001  | N/A.   |
| Nonextractable                         | 36.8  | 0.011  | N/A.   |
| <b>Muscle, thigh (TRR = 0.011 ppm)</b> |       |        |  |
| ACN                                    | 56.6  | 0.006  | N/A.   |
| Nonextractable                         | NR    | NR     | Extracted (2x) with petroleum ether:diethylether.                      |
| Petroleum ether                        | 2.0   | <0.001 | N/A.   |
| Nonextractable                         | 48.4  | 0.005  | N/A.   |

<sup>a</sup> Initial identification of prohexadione by HPLC was confirmed using 2-D TLC; initial identification of TCA in kidney by HPLC was confirmed by a second HPLC system; and Metabolite 1 in liver and egg whites were determined identical by co-elution using HPLC.

<sup>b</sup> NR = Not reported.

Table 12. Summary of radioactive residues characterized/identified in milk and tissues of laying hens orally dosed with [ $^{14}\text{C}$ ]prohexadione-calcium at 33.4 ppm for 5 consecutive days.

| Fraction                              | Egg yolk, Day 5<br>(TRR = 0.022 ppm) |              | Egg white, Day 5<br>(TRR = 0.014 ppm) |              | Kidney<br>(TRR = 0.471 ppm) |              | Liver<br>(TRR = 0.030 ppm) |              |
|---------------------------------------|--------------------------------------|--------------|---------------------------------------|--------------|-----------------------------|--------------|----------------------------|--------------|
|                                       | % TRR                                | ppm          | % TRR                                 | ppm          | % TRR                       | ppm          | % TRR                      | ppm          |
| <b>Identified <sup>a</sup></b>        |                                      |              |                                       |              |                             |              |                            |              |
| Prohexadione                          | 12.5                                 | 0.003        | 27.3                                  | 0.004        | 13.2                        | 0.062        | 14.9                       | 0.005        |
| Tricarballic acid                     | --                                   | --           | --                                    | --           | 15.5                        | 0.073        | --                         | --           |
| <b>Total identified</b>               | <b>12.5</b>                          | <b>0.003</b> | <b>27.3</b>                           | <b>0.004</b> | <b>28.7</b>                 | <b>0.135</b> | <b>14.9</b>                | <b>0.005</b> |
| <b>Characterized</b>                  |                                      |              |                                       |              |                             |              |                            |              |
| Metabolite 1                          | 2.3                                  | 0.001        | 71.5                                  | 0.010        | --                          | --           | 18.2                       | 0.006        |
| Unknown peaks                         | 13.8                                 | <0.005       | 5.1                                   | <0.002       | 5.4                         | <0.025       | 1.7                        | <0.001       |
| Aqueous                               | 12.7                                 | 0.003        | 5.6                                   | 0.001        | 6.6                         | 0.031        | 17.6                       | 0.005        |
| Petroleum ether extract               | 12.1                                 | 0.003        | 0.8                                   | <0.001       | 0.3                         | 0.001        | 2.3                        | 0.001        |
| Semi-solid gel; macromolecules        | --                                   | --           | --                                    | --           | 9.3                         | 0.044        | --                         | --           |
| Pronase hydrolysate                   | --                                   | --           | --                                    | --           | 8.4                         | 0.040        | --                         | --           |
| <b>Total identified/characterized</b> | <b>53.4</b>                          | <b>0.015</b> | <b>110.3</b>                          | <b>0.018</b> | <b>58.7</b>                 | <b>0.276</b> | <b>54.7</b>                | <b>0.018</b> |
| Nonextractable                        | 42.9                                 | 0.009        | 11.0                                  | 0.002        | 22.0                        | 0.104        | 36.8                       | 0.011        |

<sup>a</sup> See Figure 1 (Attachment II) for chemical names and structures of identified metabolites.

### Storage stability

The petitioner submitted dates indicating that extraction of egg and tissue samples were conducted within ~8 months of sample collection. Analysis dates were not provided but the petitioner stated that quantitation of residues in milk and tissues was completed within 17 months of sample collection. To demonstrate the stability of prohexadione-calcium residues over the course of the study, the petitioner conducted a storage stability study using liver samples which contained the highest residues. Residues in liver were extracted as previously described. The organosoluble extract was analyzed at the end of the study (~17 months after storage at -20 C) using 1-D TLC. The results were compared to the 2-D TLC analysis of liver conducted at the beginning of the study (4.5 months storage at -20 C). The chromatographic profiles of the methylene chloride extracts were similar demonstrating that residues of prohexadione and its metabolites are stable in hen liver for at least 17 months. Extraction flow charts, tabulated results, and chromatographic data for the storage stability samples were provided. These data are adequate to support the subject goat metabolism study.

### Study summary

The hen metabolism study is acceptable. Following oral administration of [<sup>14</sup>C]prohexadione-calcium to laying hens for five consecutive days at 8.43 ppm (34x the maximum theoretical dietary burden for poultry), the TRR in eggs and tissues each were <0.01 ppm. In the same study, following oral administration of [<sup>14</sup>C]prohexadione-calcium at 33.4 ppm (134x), the TRR were <0.008-0.019 ppm in egg yolks, <0.005-0.015 ppm in egg whites, 0.021 ppm in gizzard, 0.475-0.471 ppm in kidneys, 0.0289-0.030 ppm in liver, <0.007 ppm in breast muscle, 0.010-0.011 ppm in thigh muscle, <0.01 ppm in fat, and 0.022 ppm in skin with fat.

The levels of extractable residues in eggs and tissues of hens dosed at 33.4 ppm were mostly ≤0.01 ppm. Prohexadione was the major residue identified in egg yolks (12.5% TRR, 0.003 ppm), egg whites (27.3% TRR, 0.004 ppm), liver (14.9% TRR, 0.005 ppm); and kidney (13.2% TRR, 0.062 ppm). Tricarballic acid was identified in kidney (15.5% TRR, 0.073 ppm). An unknown component, designated as Metabolite 1, was resolved in egg whites (71.5% TRR, 0.010 ppm), egg yolks (2.3% TRR, 0.001 ppm), and liver (18.2% TRR, 0.006 ppm). Attempts to identify Metabolite 1 using a combination of techniques including acid, base, and/or enzyme hydrolysis, methylation, HPLC, GC/MS and LC/MS were unsuccessful; further attempts were not performed because of insufficient material.

### Metabolic pathway in animals

Based on the results of the goat and hen metabolism studies, the petitioner proposed that prohexadione-calcium is eventually metabolized to natural products. In ruminants, prohexadione is metabolized to despropionyl prohexadione metabolites or precursors and then to despropionyl prohexadione. The subsequent metabolism of despropionyl prohexadione then yields low molecular weight carboxylic acid and finally incorporation into naturally occurring products such as sugars, lipids and proteins. In poultry, prohexadione is metabolized to tricarballic acid and then to natural products such as proteins.

### Animal metabolism conclusions

The qualitative nature of the residue in animals is adequately understood. RAB1 will defer to the HED MARC on the toxicological significance of the prohexadione-calcium metabolites. A decision concerning which residues to regulate will then follow. A tolerance based on the parent only may not be appropriate; in such an instance a revised Section F, additional field studies, analytical methodology, and storage stability data may be needed.

### OPPTS GLN 860.1340: Residue Analytical Method - Plant Commodities

The references listed below were submitted by the petitioner in support of the registration requirements for residue analytical methods in plants.

44457803 Abdel-Baky, S. (1996) Independent Method Validation of BASF Analytical Method D9601, for Prohexadione-Calcium (BAS 125 W) in Peanuts: Lab Project Number: A008.078: 96/5199: 96127. Unpublished study prepared by Maxim Technologies, Inc.

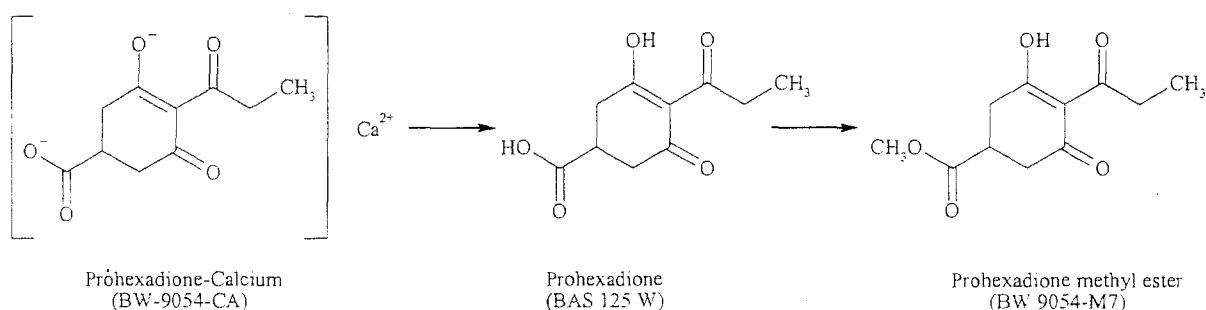
44457805 Abdel-Baky, S.; Baumann, S. (1997) Method for Determination of BAS 125 W and its Metabolite in Animal Tissues (Liver, Kidney, Fat and Muscle), Milk, and Apple Commodities: Report Amendment: Lab Project Number: 96135: 97/5034: D9608. Unpublished study prepared by Battelle and BASF Corp.

44457806 Abdel-Baky, S.; Baumann, S. (1996) Method for Determination of BAS 125 W (Prohexadione) Residues in Peanut RAC (Nutmeat and Hay), and Peanut Process Fractions (Meal and Refined Oil) by GC-MS: Lab Project Number: 95164: D9601: 96/5223. Unpublished study prepared by BASF Corp.

44725217 Hess, E.; Abdel-Baky, S.; Jones, J. (1998) Analytical Method for the HPLC/UV Determination of BW 125 31F Residues in

Plant Matrices: Lab Project Number: 98/5211: D9810. Unpublished study prepared by BASF Corporation.

To measure residues of prohexadione-calcium in plants, the petitioner has developed residue analytical methods using GC and a mass selective detector (GC/MSD). These GC/MSD methods are designated Methods D9601 and D9608. Method D9601 was first developed to measure residues of prohexadione-calcium in/on peanut nutmeat and hay. Method D9608 is identical to Method D9601, and was later developed to include instructions for the analysis of residues in/on pome fruits and animal commodities. The methods are proposed for tolerance enforcement, and were used as the data-collection methods in the analyses of samples obtained from the field, processing, and storage stability studies. Both methods were designed to measure residues of prohexadione-calcium as the prohexadione methyl ester (designated by company codes BW 125-M7 or BW9054-M7). Residues of prohexadione-calcium are calculated by multiplying a molecular weight correction factor (MWCF) of 1.107 and the quantitated residues of prohexadione methyl ester. The MWCF of 1.107 was derived by multiplying 0.938 (the MWCF from prohexadione methyl ester to prohexadione) and 1.18 (the MWCF from prohexadione to prohexadione-calcium).



To assess the significance of the oxidative metabolite BX112-I5 (which is referred to as BW125-31F in pome fruit field trials) and to assist HED in determining whether or not the compound should be included as part of the tolerance expression, the petitioner developed an HPLC/UV method, designated as Method D9810. This metabolite was identified in mature fruits (11.78% TRR, 0.0359 ppm) collected from the apple metabolism study.

#### Brief descriptions of Methods D9601/D9608

Homogenized samples of apple, apple processed commodities (wet pomace and juice), pear, peanut nutmeat and hay, and peanut processed commodity (meal) are extracted twice with acetonitrile (ACN):1.5 M sulfuric acid (9:1, v:v) and filtered with celite. Residues in/on peanut nutmeat, meal, and hay are extracted a third

time using 0.05 M sodium acetate and filtered with celite. The filtrates are combined, and water is added to adjust the volume. Residues in peanut refined oil are partitioned with hexane and ACN:1.5 M sulfuric acid (9:1, v:v). The hexane layer is discarded, and the acidic ACN layer is re-partitioned with hexane. The acidic ACN layer is collected, and the volume is adjusted with water. An aliquot of the combined filtrate or partitioned extracts (oil) is purified using a miniature chromatography column packed with ENV+ sorbant. Residues are eluted with methanol. Sulfuric acid is added, and the acidified methanol eluate is refluxed for 30 or 60 minutes. Residues are purified further using an additional Mini-ENV+ column; residues are eluted with acetone:oxalic acid for the second purification step. Residues of prohexadione-calcium are quantitated as prohexadione methyl ester (designated by company codes BW 125-M7 or BW9054-M7). Prohexadione-calcium residues are then back calculated using the molecular weight correction factor explained above. The reported LOQ for prohexadione-calcium is 0.05 ppm for all apple, pear, and peanut commodities.

#### Brief description of HPLC/UV Method D9810

Homogenized samples of apples, pears, and apple processed commodities (wet pomace and juice) are extracted twice with ACN:1.5 M sulfuric acid (9:1, v:v) and filtered (as for Method D9608). Water is added, and an aliquot of the filtrate is purified using an HLB solid-phase extraction (SPE) column. Residues are eluted with dichloromethane (DCM). The DCM eluate is reduced to dryness under vacuum, and residues are re-dissolved in 0.1% formic acid in water. Residues of BX 112-I5 are quantitated by HPLC/UV with column switching from a Betabasic Phenyl pre-column to a Kromasil 5 C18 analytical-column, and an isocratic mobile phase of methanol:water:formic acid (40:60:0.1, v:v:v). Residues of the metabolite are expressed as prohexadione (free acid) equivalents which is calculated using a molecular weight conversion factor of 1.06. The reported LOQ for BX 112-I5 was 0.05 ppm for apple and pear commodities.

#### Method validations conducted by the petitioner

The petitioner submitted validation data for Methods D9601/D9608 and D9810. Validation of Methods D9601 and D9608 was conducted using samples of processed apple wet pomace and juice (source not specified), and peanut nutmeat, hay, and processed commodities (meal and refined oil). These samples were fortified with prohexadione (free acid) and then analyzed using the above GC/MSD methods. Apparent residues of prohexadione (free acid) were less than the LOQ (<0.05 ppm) in/on two samples each of unfortified apple wet pomace and juice, and peanut nutmeat, hay, and refined oil; and in/on three unfortified samples of peanut meal.

Validation of Method D9810 was performed using samples of apples and pears (source not specified). The samples were fortified with the metabolite BX 112-I5 and analyzed using the HPLC/UV method. HED notes that the petitioner stated that a set of eight samples (including two recovery and one control samples) can be analyzed in approximately 6-7 person hours, and that a LC/MS/MS method has been developed as a confirmatory technique; however, no information or method validation data were included concerning the confirmatory method. Recoveries of fortified samples are reported in Table 13. Sample calculations and representative chromatograms were provided.

Table 13. Recoveries of prohexadione (free acid) using Methods D9601/D9608 and of metabolite BX112-I5 using Method D9810.

| Commodity                  | Fortification level, ppm | Recoveries (%) <sup>A</sup>     |
|----------------------------|--------------------------|---------------------------------|
| <b>Prohexadione</b>        |                          |                                 |
| Apple, wet pomace          | 0.05, 1.0                | 70-89 (8); <b>78</b>            |
| Apple, juice               | 0.05, 1.0                | 71-87 (8); <b>77</b>            |
| Peanut, nutmeat            | 0.05, 1.0                | 72-106 (10); <b>88</b>          |
| Peanut, hay                | 0.05, 1.0                | 61; 76-113 (7); <b>87</b>       |
| Peanut, meal               | 0.05, 1.0                | 66-69 (3); 74-85 (5); <b>75</b> |
| Peanut, refined oil        | 0.05, 1.0                | 65, 66; 70-85 (6); <b>74</b>    |
| <b>Metabolite BX112-I5</b> |                          |                                 |
| Apple                      | 0.05, 0.50               | 66, 67; 71-85 (16); <b>76</b>   |
| Pear                       | 0.05, 0.50               | 78-107 (6); <b>87</b>           |

<sup>A</sup> Each recovery value represents one sample unless otherwise noted in parentheses. Recovery values outside the acceptable 70-120% range are listed separately. Average recovery values are listed in **bold**.

#### Concurrent recoveries of Methods D9601/D9608 and D9810

In conjunction with the field, processing, and storage stability studies, the petitioner provided concurrent method recovery data. Samples of untreated apple, pear, and peanut commodities were fortified with prohexadione-calcium and analyzed concurrently with the treated field trial samples. Separate samples of untreated apple and pear commodities were fortified with the metabolite BX 112-I5 and analyzed with the treated samples. Concurrent method recovery data are presented in Table 14.

Table 14. Concurrent recoveries of prohexadione (free acid) analyzed by GC/MSD and of metabolite BX 112-15 analyzed HPLC/UV.

| Commodity (MRID)                          | MRID               | Fortification level, ppm | Recoveries (%) <sup>A</sup>        |
|---|--------------------|--------------------------|------------------------------------|
| <b>Prohexadione</b>                       |                    |                          |                                    |
| Apple                                     | 44725220           | 0.05-6.0                 | 61-68 (3); 72-113 (45); <b>86</b>  |
| Apple (processing study)                  | 44725222           | 0.05, 10.0               | 68, 90; <b>79</b>                  |
| Apple, pomace, wet                        | 44725222           | 0.05, 20.0               | 81, 107; <b>94</b>                 |
| Apple, juice                              | 44725222           | 0.05, 20.0               | 79, 86; <b>83</b>                  |
| Pear                                      | 44725221           | 0.05-1.0                 | 91-99 (5); 124-144 (3); <b>110</b> |
| Peanut, hay                               | 44457808, 44725219 | 0.05, 1.0                | 67; 72-111 (32); <b>91</b>         |
| Peanut, hay (storage stability study)     | 44457807, 44725218 | 0.05, 1.0                | 68, 69; 78-100 (10); <b>84</b>     |
| Peanut, nutmeat                           | 44457808, 44725219 | 0.05, 1.0                | 72-112 (35); <b>89</b>             |
| Peanut, nutmeat (storage stability study) | 44457807, 44725218 | 0.05, 1.0                | 69; 72-120 (11); <b>85</b>         |
| Peanut, nutmeat (processing study)        | 44457810           | 0.05, 1.0                | 90, 94; <b>92</b>                  |
| Peanut, dried nutmeat                     | 44457810           | 0.05, 1.0                | 85, 95; <b>90</b>                  |
| Peanut, meal                              | 44457810           | 0.05, 1.0                | 85, 108; <b>97</b>                 |
| Peanut, refined oil                       | 44457810           | 0.05, 1.0                | 84, 100; <b>92</b>                 |
| <b>BX 112-15</b>                          |                    |                          |                                    |
| Apple                                     | 44725220           | 0.05, 0.50               | 66, 67; 71-85 (14); <b>76</b>      |
| Apple (processing study)                  | 44725222           | 0.05, 0.50               | 73, 82; <b>78</b>                  |
| Pear                                      | 44725221           | 0.05, 0.50               | 78-107 (6); <b>87</b>              |

<sup>A</sup> Each recovery value represents one sample unless otherwise noted in parentheses. Recovery values outside the acceptable 70-120% range are listed separately. Average recovery values are listed in **bold**.

#### Specificity of Method D9601

To determine the specificity of Method D9601 in the quantitation of residues of prohexadione-calcium, other pesticide compounds registered for use on apples and peanuts were examined. Of the 199 pesticides with established tolerances on apples and/or peanuts, 174 compounds were tested using the GC/MSD method. The remaining pesticides were either not available or not GC compatible (e.g., metals or inorganic salts). Standard solutions were prepared of the pesticides of interest, and processed through the methylation and ENV+™ column purification procedures of the method. The standard solutions were then analyzed using the GC/MSD described for prohexadione (free acid). A mixture of  $\alpha$ -naphthaleneacetic acid, dioxathion, dodine, ethion, tetradifon, and ethoxyquin interfered with the quantitation of BW 9054-M7, but upon re-



injection of separately fortified apple matrices, no interferences were observed.

#### Confirmatory method

A confirmatory method is not required due to the specificity of the proposed enforcement method (GC/MSD). However, the petitioner stated that Method D9601 with a different column (DB-5, DB-1, or DB-17) may be used for the analysis of the prohexadione (free acid) in plant matrices.

#### Radiovalidation of Method D9601

To demonstrate the efficiency of the Method D9601 method in quantitating residues in aged matrices, samples from the 1993 peanut metabolism study were analyzed for residues of prohexadione using the proposed enforcement method. The results of the radiovalidation study are presented in Table 15.

Table 15. Recoveries of prohexadione in peanut samples obtained from a peanut metabolism study and analyzed using the proposed GC/MSD enforcement method.

| Commodity       | Recovery: ppm, prohexadione equivalents <sup>a</sup> |                          | Average %<br>Accountability |
|-----------------|--|--------------------------|-----------------------------|
|                 | HPLC   | Method D9601<br>(GC/MSD) |                             |
| Peanut, nutmeat | 1.43   | 1.46                     | 102                         |

<sup>a</sup> Quantitation of the prohexadione (free acid) in the metabolism study was by HPLC, while the residue GC method quantitates the prohexadione methyl ester (BW 9054-M7). The average of duplicate samples was reported.

#### Independent laboratory validation (ILV) of Method D9601

The petitioner submitted ILV data for Method D9601. The ILV was conducted by Maxim Technologies, Inc. (Middleport, NY). Peanut nutmeat was chosen as the representative matrix for the ILV because the petitioner believes that this commodity is more complex to analyze than other RACs relevant to this petition. Samples of untreated peanut nutmeat from the 1996 field trial were supplied by BASF. Samples were fortified with prohexadione (free acid) at the LOQ (0.05 ppm) and 10x the LOQ (0.50 ppm). Following fortification, samples were analyzed for residues of prohexadione using GC/MSD Method D9601. The higher fortification level represents 0.5x the proposed tolerance for peanuts (1.0 ppm). Acceptable recoveries were achieved with the first trial. The laboratory indicated that a set of seven samples could be extracted, analyzed, and data summarized in 12 person hours or ~1.5 calendar days. The results of the ILV are presented in Table 16. Apparent residues were <0.05 ppm in/on two unfortified peanut samples.

Table 16. Results of independent laboratory validation of method D9601.

| Commodity       | Fortification level, ppm | % Recovery <sup>a</sup> |
|-----------------|--------------------------|-------------------------|
| Peanut, nutmeat | 0.05                     | 78.0, 85.4              |
|                 | 0.50                     | 77.4, 99.7              |

<sup>a</sup> Each recovery value represents a single sample.

The data requirements for ILV (OPPTS GLN 860.1340) specify that validation must be conducted at the LOQ and the proposed tolerance. The submitted validation trial did not include a fortification level at the proposed tolerance. However, the concurrent and method validation studies conducted by the petitioner included fortification levels bracketing the proposed tolerances (0.6-3.0 ppm). Adequate recoveries were obtained at all fortification levels; therefore, no additional ILV data will be required to support this petition.

### Conclusions

The petitioner proposes GC/MSD methods (Methods D9601 and D9608) to measure residues of prohexadione-calcium in plants. Method D9601 was first developed to measure residues of prohexadione-calcium in/on peanut nutmeat and hay. Method D9608 is identical to Method D9601, and was later developed to include instructions for the analysis of residues in/on pome fruits and animal commodities. The methods are proposed for tolerance enforcement, and were used as the data-collection methods in the analyses of samples obtained from the field, processing, and storage stability studies.

The concurrent method recovery data indicate that GC/MSD Methods D9601/D9608 are adequate for data collection. In a specificity study, no interferences were reported when Method D9601 was tested using other pesticide compounds registered for use on apples and peanuts. Method D9601 was successfully radiovalidated using samples from a 1993 peanut metabolism study. Method D9601 was also subjected to a successful independent laboratory validation. The method and ILV have been sent to ACL for PMV (Memo, G. Kramer 8/3/99; D257929). HED will withhold a final conclusion on the adequacy of this method as an analytical enforcement method pending receipt of the PMV report.

OPPTS GLN 860.1340: Residue Analytical Method - Animal Commodities

The petitioner has submitted the reference listed below to satisfy the registration requirements for residue analytical methods in animals.

44457805 Abdel-Baky, S.; Baumann, S. (1997) Method for Determination of BAS 125 W and its Metabolite in Animal Tissues (Liver, Kidney, Fat and Muscle), Milk, and Apple Commodities: Report Amendment: Lab Project Number: 96135: 97/5034: D9608. Unpublished study prepared by Battelle and BASF Corp.

To measure residues of prohexadione-calcium and despropionyl prohexadione (also referred to as BW 125-5376) in animals, the petitioner has developed Method D9608. Quantitation of prohexadione-calcium is by GC/MSD, and quantitation of the despropionyl metabolite is by HPLC/UV. It is noted that in the ruminant metabolism study, metabolites related to, or a precursor of, despropionyl prohexadione were identified in kidney (31.7% TRR, 0.980 ppm) and liver (10.5% TRR, 0.045 ppm). Method D9608 is proposed for tolerance enforcement, and was used as the data-collection methods in the analyses of samples obtained from ruminant feeding study.

Brief descriptions of Method D9608 for the analysis of prohexadione-calcium

Homogenized samples of bovine liver, kidney, fat, and muscle and samples of milk are extracted with acetonitrile (ACN):1.5 M sulfuric acid (9:1, v:v). The extracts are then purified using Mini-ENV+™ columns as described earlier for pome fruits. Residues of prohexadione-calcium are calculated by multiplying a molecular weight correction factor (MWCF) of 1.107 and the quantitated residues of prohexadione methyl ester. The reported LOQ for prohexadione-calcium was 0.05 ppm for liver, kidney, fat, and muscle and 0.01 ppm for milk.

Brief descriptions of Method D9608 for the analysis of despropionyl prohexadione

An aliquot of extracted bovine liver and kidney samples from the acidic ACN extraction procedure is evaporated to remove the ACN and hydrolyzed with 6 N HCl at reflux for 30 minutes. Residues are purified through a mini-SAX column; the load and acidic water rinse are collected. Residues are further purified through a mini-Carbopack B column; residues are eluted with 0.1 N sodium hydroxide. The pH of the eluate is reduced to <2 with sulfuric acid, and the residues are purified again using mini-isolute ENV+™ column chromatography. Residues are eluted with acetone, evaporated to dryness under nitrogen, and re-dissolved in water for HPLC analysis. The HPLC system utilizes UV detection (265 nm) and

column switching with an initial Hypersil C18 column and isocratic mobile phase of water:acetic acid (99:1, v:v), and second ODS-AQ column and isocratic mobile phase of ACN:water:acetic acid (3:96:1, v:v:v). The reported LOQ for despropionyl prohexadione was 0.05 ppm for liver and kidney.

#### Method validation conducted by the petitioner

During development of Method D9608, samples of bovine tissues and milk (source not specified) were fortified with prohexadione (free acid) and analyzed using GC/MSD. Separate samples of bovine liver and kidney were fortified with the despropionyl metabolite and analyzed using HPLC/UV. Apparent residues of prohexadione (free acid) were less than the LOQ (<0.05 ppm) in two samples each of unfortified bovine tissues (fat, muscle, kidney, and liver) and less than the LOQ (<0.01 ppm) in two samples of milk. Apparent residues of despropionyl metabolite were less than the LOQ (<0.05 ppm) in two samples each of unfortified bovine kidney and liver. Recoveries of fortified samples are reported in Table 17; sample calculations and chromatograms were included.

Table 17. Recoveries of prohexadione (free acid) and its despropionyl metabolite using Method D9608. (Quantitation of prohexadione-is by GC/MSD, and quantitation of despropionyl metabolite is by HPLC/UV.)

| Commodity                        | Fortification level, ppm | Recoveries (%) <sup>a</sup>         |
|----------------------------------|--------------------------|-------------------------------------|
| <b>Prohexadione</b>              |                          |                                     |
| Fat                              | 0.05, 1.0                | 69; 76-93 (7); <b>80</b>            |
| Liver                            | 0.05, 1.0                | 72-89 (8); <b>80</b>                |
| Kidney                           | 0.05, 1.0                | 76-105 (8); <b>87</b>               |
| Muscle                           | 0.05, 1.0                | 72-91 (8); <b>79</b>                |
| Milk                             | 0.01, 1.0                | 83-95 (8); <b>89</b>                |
| <b>Despropionyl prohexadione</b> |                          |                                     |
| Liver                            | 0.05, 1.0                | 84-102 (3); 123-125 (4); <b>112</b> |
| Kidney                           | 0.05, 1.0                | 91-120 (5); 122-129 (3); <b>111</b> |

<sup>a</sup> Each recovery value represents one sample unless otherwise noted in parentheses. Recovery values outside the acceptable 70-120% range are listed separately. Average recovery values are listed in **bold**.

#### Concurrent recoveries of Method D9608

In conjunction with the ruminant feeding study, the petitioner provided concurrent method recovery data. Samples of untreated bovine commodities were fortified with prohexadione and analyzed concurrently with the treated feeding study samples. Separate samples of untreated bovine liver and kidney were fortified with the despropionyl metabolite and analyzed with the treated samples. Concurrent recovery data are presented in Table 18.

Table 18. Concurrent method recoveries of prohexadione (free acid) and despropionyl metabolite analyzed by Method D9608. (Quantitation of prohexadione-calcium is by GC/MSD, and quantitation of the despropionyl metabolite is by HPLC/UV.)

| Commodity                          | Fortification level, ppm | Recoveries (%) <sup>a</sup>         |
|------------------------------------|--------------------------|-------------------------------------|
| <b>Prohexadione</b>                |                          |                                     |
| Milk (whole, cream, and skim milk) | 0.01, 1.0                | 66, 69; 70-102 (31); <b>81</b>      |
| Fat                                | 0.05, 1.0                | 77-95 (4); <b>85</b>                |
| Kidney                             | 0.05-5.0                 | 76-119 (6); <b>92</b>               |
| Liver                              | 0.05, 1.0                | 65, 69; 77, 87; <b>75</b>           |
| Muscle                             | 0.05, 1.0                | 84-86 (4); <b>85</b>                |
| <b>Despropionyl prohexadione</b>   |                          |                                     |
| Kidney                             | 0.05-1.0                 | 98-116 (3); 121-131 (3); <b>117</b> |
| Liver                              | 0.05, 1.0                | 115; 123-132 (3); <b>125</b>        |

<sup>a</sup> Each recovery value represents one sample unless otherwise noted in parentheses. Recovery values outside the acceptable 70-120% range are listed separately. Average recovery values are listed in **bold**.

#### Specificity of Method D9608

To determine the specificity of Method D9608 in the quantitation of residues of despropionyl metabolite, other pesticide compounds registered for use on meat (kidney and liver) were examined. Twenty-two pesticides with established tolerances on meat (kidney and liver) were tested using the HPLC/UV method. Standard solutions were prepared of the pesticides of interest, and processed through the methylation and column purification procedures of the method. The standard solutions were then analyzed using the HPLC/UV method described for the despropionyl metabolite. No interferences were observed.

#### Confirmatory methods

A confirmatory method is not required due to the specificity of the proposed enforcement method (GC/MSD). However, the petitioner stated that Method D9601 with a different column (DB-5, DB-1, or DB-17) may be used for the analysis of the prohexadione (free acid) in plant matrices.

#### Radiovalidation of Method D9608

To demonstrate the efficiency of the method in quantitating residues in aged matrices, kidney samples from the goat metabolism study were analyzed for residues of prohexadione (free acid) and its despropionyl metabolite. During the conduct of the goat metabolism study, two extraction profiles were performed on goat kidney. The second profile was conducted after 28 months of frozen storage and coincided with the radiovalidation study; therefore, the results of the second extraction profile were used for

comparison purposes. Untreated bovine kidney was used for control and fortified method recovery samples. The results of the radiovalidation study are presented in Table 19.

Table 19. Recoveries of prohexadione and its despropionyl metabolite in goat kidney obtained from a goat metabolism study and analyzed using the proposed enforcement method (D9608).

| Analyte                    | Recovery; ppm, expressed as prohexadione equivalents <sup>a</sup> |                   | Average % Accountability |
|----------------------------|---|-------------------|--------------------------|
|                            | HPLC  | GC/MSD or HPLC/UV |                          |
| Prohexadione (parent acid) | 0.2   | 0.132             | 66                       |
| Despropionyl prohexadione  | 0.64  | 0.21              | 33                       |

<sup>a</sup> Average of duplicate samples; values were corrected for average method recovery.

#### ILV of Method D9608

The petitioner submitted ILV data for Method D9608. The ILV was conducted by Maxim Technologies, Inc. (Middleport, NY). Bovine kidney was chosen as the representative matrix, and was fortified with prohexadione (free acid) and despropionyl metabolite each at the LOQ (0.05 ppm) and at the level of expected residues (0.5 ppm for prohexadione and 0.1 ppm for the despropionyl metabolite). Because milk is a commonly monitored commodity, this matrix was also fortified with prohexadione (free acid) at the LOQ (0.01 ppm) and at 2x the LOQ (0.02 ppm). Samples of control bovine kidney and milk were supplied by BASF. Following fortification, samples were analyzed using Method D9608.

The testing laboratory had communications with the petitioner concerning clarifications of the method description during the column-elution profile phase (prior to initiating analysis of the first trial with kidney). Acceptable recoveries were achieved with the first trial for both kidney and milk. The laboratory indicated that a set of seven samples of kidney could be extracted in 24 person hours or ~1.5 calendar days; preparation of samples for GC and HPLC analyses requires two persons eight hours or 16 person hours. A set of seven samples of milk could be extracted in 12 person hours or ~1.5 calendar days; preparation of samples for GC analysis requires eight hours. GC and HPLC analyses then proceeded overnight with data reduction the following day.

The results of the ILV are presented in Table 20. Apparent residues of prohexadione and its metabolite BW 125-5376 were each <0.05 ppm in/on two unfortified bovine kidney samples. Apparent residues of prohexadione were <0.01 ppm in/on two unfortified milk samples. Adequate recoveries were obtained at all fortification levels; therefore, no additional ILV data will be required to support this petition.

Table 20. Results of independent laboratory validation of method D9608.

| Commodity | Fortification level, ppm | % Recovery <sup>a</sup> |                           |
|-----------|--------------------------|-------------------------|---------------------------|
|           |                          | Prohexadione            | Despropionyl prohexadione |
| Kidney    | 0.05                     | 80.4, 80.8              | 65.4, 79.3                |
|           | 0.1                      | --                      | 92.7, 98.4                |
|           | 0.5                      | 81.7, 87.3              | --                        |
| Milk      | 0.01                     | 86.1, 88.6              | --                        |
|           | 0.02                     | 81.0, 86.0              | --                        |

<sup>a</sup> Each recovery value represents one sample unless otherwise indicated in parentheses.

### Conclusions

Method D9608 was used as the data-collection method in the analyses of samples obtained from ruminant feeding study. The concurrent method recovery data indicate that Method D9608 is adequate for data collection. In a specificity study, no interferences were reported when Method D9608 was tested using other pesticide compounds with established tolerances on meat (kidney and liver). Method D9608 was successfully radiovalidated using samples from a goat metabolism study. The results of the independent laboratory validation was also successful. The method and ILV have been sent to ACL for PMV (Memo, G. Kramer 8/3/99; D257929). HED will withhold a final conclusion on the adequacy of this method as an analytical enforcement method pending receipt of the PMV report.

### OPPTS GLN 860.1360: Multiresidue Method

The petitioner submitted data (citation listed below) concerning the recovery of residues of prohexadione and its despropionyl metabolite (BW 125-5376) using FDA multiresidue method protocols (PAM Vol. I). These data will be forwarded to FDA for evaluation.

44457802 Fomenko, J. (1996) PAM I Multiresidue Testing for BAS 125 W and its Metabolite: Lab Project Number: 96083: A008.077: 96/5196. Unpublished study prepared by Maxim Technologies, Inc.

Recovery of prohexadione and the despropionyl metabolite was unsuccessful using FDA multiresidue method protocols. Prohexadione, despropionyl metabolite, and their corresponding methyl esters did not produce sufficient responses using electron capture detection under Protocol C. Therefore, no further testing was performed under Protocols B, D, E, or F. Prohexadione and the despropionyl metabolite BW 125-5376 could not be evaluated by Protocol A because an N-methylcarbamate structure is not present in either of these compounds.

## OPPTS GLN 860.1380: Storage Stability Data

### Storage conditions and intervals of samples

*Peanut field trials:* Peanut plants were dug and inverted two to eight days prior to collection and allowed to dry to typical moisture levels in the field. After drying, peanut nutmeat and hay samples were collected. The peanut RAC samples were placed in coolers with blue ice and frozen within 4 hours of harvest. Samples were then shipped frozen to the analytical laboratory, BASF Agricultural Products Center (APC; Research Triangle Park, NC), where samples remained frozen (<-10 C) until sample preparation. Samples were homogenized and stored frozen in plastic bags for 11-391 days (1995 peanut trials) or 4-55 days (1997 peanut trials) prior to analysis.

*Pome fruit field trials:* The harvested apple and pear samples were placed in coolers with blue ice and frozen within 4 hours of harvest. Samples were then shipped frozen to the analytical laboratory where samples remained frozen (<-10 C) until sample preparation. Samples were homogenized and stored frozen in plastic bags for 1-82 days (apples) or 7-59 days (pears) until analysis. Apple and pear samples analyzed for the metabolite BX 112-15 were stored frozen for 48-807 days (~2-27 months) prior to analysis.

*Apple processing study:* Whole apples were bagged and placed into coolers with ice. Samples were hand delivered to the processing facility (Wm. J. Englar and Associates, Inc., Moses Lake, WA) and frozen within 4 hours of harvest. Whole apples were processed on the day of receipt. At the processing facility, the collected samples of apples (unwashed and washed), wet pomace, and juice were frozen and shipped within 3 days by overnight delivery (FedEx) to APC. At APC, samples were frozen (<-10 C) until they were homogenized; once homogenized for consistency, samples were frozen until residue analysis.

*Peanut processing study:* Peanut plants were dug by hand and inverted for field drying for 5-7 days. Peanuts were mechanically collected using a combine and spread on a tarp. Replicate untreated and 5x-treated samples were randomly collected, bagged, and frozen. Samples were delivered within 2-11 days of harvest, on dry ice via FedEx to the processing facility (Food Protein Research and Development Center, Texas A&M University, Bryan, TX). Samples were stored frozen 197 days (~6.5 months) prior to processing. At the processing facility, the collected RAC, dried nutmeat, meal, and refined oil samples were frozen and shipped on the day processing was completed by overnight delivery (FedEx) to APC. At APC, samples were frozen (<-20 C) until being homogenized; once homogenized for consistency, samples were frozen until residue analysis.



*Ruminant feeding study:* Milk samples were frozen immediately following collection and shipped frozen to the analytical laboratory on a weekly basis. Samples of fat, kidney, liver, and muscle collected after sacrifice were immediately frozen and shipped frozen to the analytical laboratory. Samples were stored frozen (<-10 C) until analysis. Initial analyses were completed approximately within 1 month of sampling.

The freezer storage intervals of samples are presented in Table 21.

Table 21. Storage intervals of samples from the submitted field trial, processing, and feeding studies.

| Commodity                      | MRID     | Storage Interval  |
|--------------------------------|----------|---|
| <b>Plant Commodities</b>       |          |   |
| Apple                          | 44725220 | 5-100 days (~0-3 months)  |
| Apple (processing study)       | 44725222 | 33-34 days (~1 month) prohexadione<br>776 days (~26 months) BX 112-15 |
| Apple, pomace, wet             | 44725222 | 33 days (~1 month)  |
| Apple, juice                   | 44725222 | 33 days (~1 month)  |
| Pear                           | 44725221 | 45-101 days (~1-3 months)   |
| Peanut, nutmeat                | 44457808 | 155-204 days (~5-7 months)  |
|                                | 44725219 | 10-67 days (~0-2 months)  |
| Peanut, hay                    | 44457808 | 150-200 days (~5-7 months)  |
|                                | 44725219 | 14-55 days (~0-2 months)  |
| Peanut (processing study)      | 44457810 | 411 days (~14 months)   |
| Peanut, dried nutmeat          | 44457810 | 212 days (~7 months)  |
| Peanut, meal                   | 44457810 | 211 days (~7 months)  |
| Peanut, refined oil            | 44457810 | 212 days (~7 months)  |
| <b>Animal Commodities</b>      |          |   |
| Cow, milk (whole, cream, skim) | 44457809 | within 1 month of collection  |
| Cow, fat                       | 44457809 |   |
| Cow, kidney                    | 44457809 |   |
| Cow, liver                     | 44457809 |   |
| Cow, muscle                    | 44457809 |   |

## Storage stability data - plant commodities

In support of the storage intervals and conditions of samples from the peanut field trials, BASF submitted the following studies (citations listed below) investigating the frozen storage stability of weathered residues of prohexadione-calcium in/on peanut hay and nutmeat.

44457807 Wofford, J.; Baumann, S.; Riley, M. (1997) Storage Stability of Prohexadione-Calcium Residues in Peanuts Resulting from Field Applications of BAS 125 W (Nine Months Storage): Lab Project Number: 96119: 97/5340: D9601. Unpublished study prepared by BASF Corp., Agvise Laboratories, Inc. and Agricultural Systems Assoc.

44725218 Wofford, J.; Abdel-Baky, S.; Riley, M. (1998) Storage Stability of Prohexadione-Calcium Residues in Peanuts Resulting from Field Applications of BAS 125 W (Twelve Months Storage): Lab Project Number: 98/5052: 96119: 96-3423. Unpublished study prepared by BASF Corporation.

Samples of peanut hay and nutmeat bearing weathered residues were homogenized, stored frozen (<-10 C) in plastic bags, and analyzed at various intervals for up to 12 months. Samples used in this study were obtained from the NC field study. Samples were analyzed for residues of prohexadione-calcium (quantitated as the prohexadione methyl ester) using Method D9601 described under "OPPTS GLN 860.1340: Residue Analytical Method-Plant Commodities" section. The reported limits of quantitation (LOQ) and detection (LOD) were 0.05 ppm and 0.02 ppm, respectively, for peanut commodities. Control samples were fortified with prohexadione-calcium at 0.05 and 1.0 ppm at the time of analysis for concurrent recoveries. The average method recoveries were 85% for fortified nutmeat and 84% for fortified hay. Concurrent recovery data of samples from the storage stability study are presented in Table 14. Apparent residues of prohexadione-calcium were each less than the LOQ in/on six untreated samples each of peanut hay and nutmeat. The results of storage stability study using weathered residues are presented in Table 22.

Table 22. Storage stability of residues of prohexadione-calcium from peanut hay and nutmeat samples bearing weathered residues and stored frozen (<-10 C).

| Matrix         | Treatment Rate<br>(Total Seasonal) | Measurement                    | Storage Interval (months)         |                          |                 |                          |                 |                 |
|----------------|------------------------------------|--------------------------------|-----------------------------------|--------------------------|-----------------|--------------------------|-----------------|-----------------|
|                |                                    |                                | 0                                 | 1                        | 3               | 6                        | 9               | 12              |
| Peanut Hay     | 0.96 lb ai/A                       | Residue (ppm) <sup>a</sup>     | 0.420<br>0.449<br>0.473<br>(0.45) | 0.350<br>0.495<br>(0.42) | 0.397<br>(0.40) | 0.385<br>0.401<br>(0.39) | 0.404<br>(0.40) | 0.493<br>(0.49) |
|                |                                    | Relative Recovery <sup>b</sup> | --                                | 93%                      | 89%             | 87%                      | 89%             | 109%            |
|                |                                    | % Decline <sup>c</sup>         | --                                | none                     | 11%             | 13%                      | 11%             | none            |
|                | 1.63 lb ai/A                       | Residue (ppm) <sup>a</sup>     | 0.842<br>0.887<br>0.897<br>(0.88) | 0.721<br>0.844<br>(0.78) | 0.772<br>(0.77) | 0.808<br>0.818<br>(0.81) | 0.954<br>(0.95) | 0.757<br>(0.76) |
|                |                                    | Relative Recovery <sup>b</sup> | --                                | 89%                      | 88%             | 92%                      | 108%            | 86%             |
|                |                                    | % Decline <sup>c</sup>         | --                                | 11%                      | 12%             | none                     | none            | 14%             |
| Peanut Nutmeat | 0.96 lb ai/A                       | Residue (ppm) <sup>a</sup>     | 0.593<br>0.600<br>0.620<br>(0.60) | 0.415<br>0.535<br>(0.48) | 0.277<br>(0.28) | 0.341<br>0.352<br>(0.35) | 0.382<br>(0.38) | 0.399<br>(0.40) |
|                |                                    | Relative Recovery <sup>b</sup> | --                                | 80%                      | 47%             | 58%                      | 63%             | 67%             |
|                |                                    | %Decline <sup>c</sup>          | --                                | 20%                      | 53%             | 42%                      | 37%             | 33%             |
|                | 1.63 lb ai/A                       | Residue (ppm) <sup>a</sup>     | 0.731<br>0.740<br>0.757<br>(0.74) | 0.557<br>0.568<br>(0.56) | 0.371<br>(0.37) | 0.407<br>0.431<br>(0.42) | 0.517<br>(0.52) | 0.516<br>(0.52) |
|                |                                    | Relative Recovery <sup>b</sup> | --                                | 76%                      | 50%             | 57%                      | 70%             | 70%             |
|                |                                    | %Decline <sup>c</sup>          | --                                | 24%                      | 50%             | 43%                      | 30%             | 30%             |

<sup>a</sup> Either average residue values of multiple determinations or the rounded value of a single analysis are reported in parentheses.

<sup>b</sup> Relative recovery was calculated by dividing the average residues with the 0-day average residues.

<sup>c</sup> Uncorrected percent decline was determined using the average 0-day residue value; only declines >10% were considered significant.

## Conclusions

The maximum storage intervals (from harvest to analysis for residues of prohexadione-calcium) of samples from the field and processing studies were as follows: apples and pears (3 months), peanut hay and nutmeat (7 months), apple juice and wet pomace (1 month), and peanut meal and refined oil (7 months; 6.5 months as the RAC homogenate and <1 month as the processed commodity). The maximum storage interval (from harvest to residue analysis of metabolite BX 112-15) of samples of apples from the processing study was 26 months.

Storage stability data are available for peanut nutmeat and hay. These data indicate that weathered residues of prohexadione-calcium are relatively stable in/on peanut hay when stored under frozen conditions for up to 12 months. In peanut nutmeat, residues of prohexadione-calcium initially declined (~50%) between 1- and 3-month intervals but stabilized (30-43% residue decline) at the 6-, 9-, and 12-month intervals. Provided that field trial residues are adjusted to account for residue decline in peanut nutmeat during storage, these data support the storage intervals of samples collected from the peanut field trials.

According to the petitioner, no storage stability data were submitted for pome fruits because initial analyses of field samples were completed within one month. HED, however, notes that some residues reported in the pome field trials include values for samples which were re-analyzed after 3 months of storage. Given the observed instability of prohexadione-calcium residues in/on peanut nutmeat, the petitioner is required to provide confirmatory storage stability data on pome fruits reflecting an interval of three months. The petitioner has indicated that a freezer storage stability study for prohexadione-calcium and the metabolite BX-112-15 in apples has been initiated and is scheduled to be completed by November 2000.

No storage stability data are required for the processed commodities of apples because analyses of samples were completed within one month of sampling. However, storage stability data on the processed commodities of peanuts, reflecting an interval of 7 months, are required.

No storage stability data are required for milk and ruminant tissues because analyses of samples, collected from the ruminant feeding study, were conducted approximately within one month of sampling.

## OPPTS GLN 860.1500: Crop Field Trials

### Peanuts

BASF submitted two volumes of peanut field trial data (citations listed below) to support the establishment of the proposed tolerances for residues of prohexadione-calcium in/on peanut commodities.

44457808 Wofford, J.; Abdel-Baky, S.; Riley, M. (1996) Magnitude of Prohexadione Residues in Peanuts: Lab Project Number: 94157: 96/5206: ER 96007. Unpublished study prepared by BASF Corp.

44725219 Wofford, J.; Abdel-Baky, S.; Riley, M. et al. (1998) Magnitude of Prohexadione-Calcium Residues in Peanuts: Lab Project Number: 97077: 98/5054: 97-2010. Unpublished study prepared by BASF Corporation.

In the first study (MRID 44457808), a total of 12 peanut field trials were conducted during the 1995 growing season in AL(1), FL(1), GA(4), NC(2), OK(1), SC(1), and TX(2). Because storage stability data demonstrated that approximately 50% of residues were lost after 3 months storage and the storage intervals for the 1995 trials were ~7 months, the peanut field trial studies were repeated in 1997. The second study was designed to have peanut samples analyzed within 30 days of harvest in order to minimize residue degradation. In the second study (MRID 44725219), a total of 12 peanut field trials were conducted during the 1997 growing season in AL(1), FL(1), GA(4), NC(2), OK(2), SC(1), and TX(1). For both studies, peanut plants received three broadcast foliar applications, with 20- to 22-day retreatment intervals, of the 75% DF formulation with 1 quart/A each of crop oil concentrate and UAN (30%) at 0.125 lb ai/A/application (0.375 lb ai/A/season; 1x the maximum proposed seasonal application rate). An additional plot at each site was left untreated and served as a control. Applications were made in spray volumes of 10-23 gal/A using ground equipment to simulate commercial application.

The RAC samples, including nutmeat and hay, were collected 25 days (including drying time) following the last application. Plants were dug and inverted to field dry for 2-8 days. The moisture level of harvested peanut hay was monitored in efforts to simulate normal agricultural practices. Percent moisture was determined by the difference between wet and dry weights following oven drying for 18 hours at 100 C. Moisture levels ranged 25-58% in the 1995 trial samples and 15-27% in the 1997 trial samples. Peanut hull samples were also collected in the 1995 field trials, however, these samples were not analyzed because HED no longer considers peanut hulls a significant livestock feed item (OPPTS 860.1000, Table 1).

Residue decline studies were part of the 1995 and 1997 field studies. For the 1995 decline study, samples of peanut hay and nutmeat were harvested at 17-, 32-, and 39-day posttreatment intervals (PTIs) in one field trial conducted in NC. For the 1997 decline study, samples of peanut hay and nutmeat were harvested at 15-, 35-, and 45-day PTIs in one field trial conducted in GA. The additional samples collected in two decline trials were within -10 or -8, 7 or 10, and 14 or 20 days of the proposed PHI (nominal harvest).

Single control and replicate treated samples were collected by hand from each test plot. Samples were placed in coolers with blue ice and frozen within 4 hours of harvest. All samples were shipped frozen to the analytical laboratory, BASF Agricultural Products Center (APC; Research Triangle Park, NC), where samples were stored frozen (<-10 C) until analysis. Total storage intervals for samples from the peanut field trials are presented in Table 21.

Samples of nutmeat and hay were homogenized with dry ice at APC and stored frozen in plastic bags until analysis. Homogenized samples were analyzed for residues of prohexadione-calcium (quantitated as the prohexadione methyl ester) using Method D9601. The reported LOQ for prohexadione-calcium was 0.05 ppm for all peanut commodities. Apparent residues of prohexadione-calcium were below the LOQ (<0.05 ppm) in/on 31 samples each of untreated nutmeat and hay. Residues in/on treated nutmeat and hay samples from the 1995 and 1997 studies are presented in Tables 23a and 23b, respectively.

Table 23a. Residues of prohexadione-calcium in/on peanut commodities from a 1995 study with three broadcast foliar applications of the 75% DF formulation at 0.125 lb ai/A/application (0.375 lb ai/A/season: 1x).

| County, State (EPA Region)<br>Study Number | PHI<br>(days) | Prohexadione (free acid)<br>Residues (ppm) | Prohexadione-Calcium<br>Residues (ppm) <sup>a</sup> |
|--|---------------|--|---|
| <b>Peanut, nutmeat</b>                     |               |  |   |
| Henry, AL (Region 2) 95119                 | 25            | <0.05, <0.05                               | <0.05, <0.05  |
| Jackson, FL (Region 3) 95120               | 25            | <0.05, 0.162                               | <0.05, 0.191  |
| Macon, GA (Region 2) 95115                 | 25            | 0.298 <sup>b</sup> , 0.318                 | 0.352, 0.375  |
| Macon, GA (Region 2) 95116                 | 25            | 0.216, 0.223                               | 0.255, 0.263  |
| Mitchell, GA (Region 2) 95117              | 25            | 0.077, 0.078                               | 0.091, 0.092  |
| Mitchell, GA (Region 2) 95118              | 25            | 0.057, 0.060                               | 0.067, 0.071  |
| Wayne, NC (Region 2) 95112                 | 17            | 0.370 <sup>b</sup> , 0.388 <sup>b</sup>    | 0.437, 0.458  |
|  | 25            | 0.291, 0.301                               | 0.343, 0.355  |
|  | 32            | 0.156, 0.221                               | 0.184, 0.261  |
|  | 39            | 0.085, 0.119                               | 0.100, 0.140  |
| Pender, NC (Region 2) 95113                | 25            | 0.220, 0.279                               | 0.260, 0.329  |
| Caddo, OK (Region 6) 95121                 | 25            | 0.073, 0.144                               | 0.086, 0.170  |
| Barnwell, SC (Region 2) 95114              | 25            | 0.259, 0.287 <sup>b</sup>                  | 0.306, 0.339  |
| Hockley, TX (Region 8) 95122               | 25            | <0.05, 0.075                               | <0.05, 0.089  |
| Waller, TX (Region 6) 95123                | 25            | 0.165, 0.187                               | 0.195, 0.221  |
| <b>Peanut, hay</b>                         |               |  |   |
| Henry, AL (Region 2) 95119                 | 25            | 0.071, 0.088                               | 0.084, 0.104  |
| Jackson, FL (Region 3) 95120               | 25            | 0.053, 0.054                               | 0.063, 0.064  |
| Macon, GA (Region 2) 95115                 | 25            | 0.072 <sup>b</sup> , 0.073                 | 0.085, 0.086  |
| Macon, GA (Region 2) 95116                 | 25            | 0.157, 0.157                               | 0.185, 0.185  |
| Mitchell, GA (Region 2) 95117              | 25            | 0.059, 0.062                               | 0.070, 0.073  |
| Mitchell, GA (Region 2) 95118              | 25            | 0.179, 0.209 <sup>b</sup>                  | 0.211, 0.247  |
| Wayne, NC (Region 2) 95112                 | 17            | 0.133, 0.133                               | 0.157, 0.157  |
|  | 25            | 0.154, 0.180                               | 0.182, 0.212  |
|  | 32            | 0.069, 0.077                               | 0.081, 0.091  |
|  | 39            | 0.054, 0.071 <sup>b</sup>                  | 0.064, 0.084  |
| Pender, NC (Region 2) 95113                | 25            | 0.271 <sup>b</sup> , 0.278 <sup>b</sup>    | 0.320, 0.328  |
| Caddo, OK (Region 6) 95121                 | 25            | 0.070, 0.110                               | 0.083, 0.130  |
| Barnwell, SC (Region 2) 95114              | 25            | 0.073, 0.080                               | 0.086, 0.094  |
| Hockley, TX (Region 8) 95122               | 25            | 0.122, 0.138                               | 0.144, 0.163  |
| Waller, TX (Region 6) 95123                | 25            | <0.05, <0.05                               | <0.05, <0.05  |

<sup>a</sup> Prohexadione-calcium residues were calculated by the study reviewer using a MWCF of 1.18 (acid to calcium).

<sup>b</sup> The highest residue value of replicate injections is reported.

Table 23b. Residues of prohexadione-calcium in/on peanut commodities from a 1997 study with three broadcast foliar applications of the 75% DF formulation at 0.125 lb ai/A/application (0.375 lb ai/A/season: 1x).

| County, State (EPA Region)<br>Study Number | PHI<br>(days) | Prohexadione (free acid)<br>Residues (ppm)   | Prohexadione-Calcium<br>Residues (ppm) <sup>a</sup> |
|--|---------------|--|---|
| <b>Peanut, nutmeat</b>                     |               |  |   |
| Barbour, AL (Region 2) 97216               | 25            | 0.073, 0.085   | 0.086, 0.100  |
| Jackson, FL (Region 3) 97217               | 25            | <0.05, <0.05   | <0.05, <0.05  |
| Macon, GA (Region 2) 97212                 | 25            | 0.237 <sup>b</sup> , 0.304 <sup>b</sup>  | 0.280, 0.359  |
| Macon, GA (Region 2) 97213                 | 25            | <0.05, 0.058 <sup>b</sup>  | <0.05, 0.068  |
| Tift, GA (Region 2) 97214                  | 15            | 0.140 <sup>b</sup> , 0.168 <sup>b</sup>  | 0.165, 0.198  |
|  | 25            | <0.05, <0.05   | <0.05, <0.05  |
|  | 35            | <0.05, <0.05   | <0.05, <0.05  |
|  | 45            | <0.05, <0.05   | <0.05, <0.05  |
| Turner, GA (Region 2) 97215                | 25            | 0.083, 0.091   | 0.098, 0.107  |
| Sampson, NC (Region 2) 97209               | 25            | <0.05, <0.05   | <0.05, <0.05  |
| Sampson, NC (Region 2) 97210               | 25            | 0.068, 0.072   | 0.080, 0.085  |
| Caddo, OK (Region 6) 97218                 | 25            | 0.115 <sup>b</sup> , 0.127 <sup>b</sup>  | 0.136, 0.150  |
| Washita, OK (Region 6) 97219               | 25            | <0.05, <0.05   | <0.05, <0.05  |
| Barnwell, SC (Region 2) 97211              | 25            | 0.628 <sup>b</sup> , 0.629 <sup>b</sup> , 0.653 <sup>b</sup> ,<br>0.759 <sup>b</sup> | 0.741, 0.742, 0.771, <b>0.896</b>                   |
| Hockley, TX (Region 8) 97220               | 25            | 0.094, 0.103   | 0.111, 0.122  |
| <b>Peanut, hay</b>                         |               |  |   |
| Barbour, AL (Region 2) 97216               | 25            | <0.05, <0.05   | <0.05, <0.05  |
| Jackson, FL (Region 3) 97217               | 25            | <0.05, <0.05   | <0.05, <0.05  |
| Macon, GA (Region 2) 97212                 | 25            | 0.133, 0.144 <sup>b</sup>  | 0.157, 0.170  |
| Macon, GA (Region 2) 97213                 | 25            | 0.102, 0.133   | 0.120, 0.157  |
| Tift, GA (Region 2) 97214                  | 15            | 0.647, 0.955 <sup>b</sup>  | 0.763, 1.127  |
|  | 25            | 0.107, 0.166   | 0.126, 0.196  |
|  | 35            | <0.05, <0.05   | <0.05, <0.05  |
|  | 45            | <0.05, <0.05   | <0.05, <0.05  |
| Turner, GA (Region 2) 97215                | 25            | 0.103, 0.127   | 0.122, 0.150  |
| Sampson, NC (Region 2) 97209               | 25            | 0.112, 0.118   | 0.132, 0.139  |
| Sampson, NC (Region 2) 97210               | 25            | <0.05, <0.05   | <0.05, <0.05  |
| Caddo, OK (Region 6) 97218                 | 25            | 0.131, 0.142   | 0.155, 0.168  |
| Washita, OK (Region 6) 97219               | 25            | 0.064, 0.103   | 0.076, 0.122  |
| Barnwell, SC (Region 2) 97211              | 25            | 0.326 <sup>b</sup> , 0.384 <sup>b</sup> , 0.400 <sup>b</sup> ,<br>0.457 <sup>b</sup> | 0.385, 0.453, 0.472, <b>0.539</b>                   |
| Hockley, TX (Region 8) 97220               | 25            | 0.142, 0.157   | 0.168, 0.185  |

<sup>a</sup> Prohexadione-calcium residues were calculated by the study reviewer using a MWCF of 1.18 (acid to calcium).

<sup>b</sup> The highest residue value of replicate injections is reported.



Geographic representation of residue data for peanuts is adequate. According to Tables 1 and 5 of OPPTS 860.1500, 12 trials should be conducted for peanuts in Regions 2 (8 trials), 3 (1 trial), 6 (2 trials), and 8 (1 trial). In 1995, twelve field trials for peanuts were conducted in Regions 2 (8 trials; AL, GA, NC, SC), 3 (1 trial; FL), 6 (2 trials; OK and TX), and 8 (1 trial; TX). In 1997, an additional twelve field trials for peanuts were conducted in Regions 2 (8 trials; AL, GA, NC, and SC), 3 (1 trial; FL), 6 (2 trials; OK), and 8 (1 trial; TX).

### Conclusions

Residues of prohexadione-calcium were <0.05-0.896 ppm in/on peanut nutmeat and <0.05-0.539 ppm in/on peanut hay harvested 25 days (the proposed PHI) following the last of three broadcast foliar applications of the 75% DF formulation at 0.125 lb ai/A/application (0.375 lb ai/A/season; 1x). The 1997 residue decline data indicate that residues of prohexadione-calcium dissipate with longer preharvest intervals. Residues were highest in the 15-day PHI and declined to the lowest levels at the 25-, 35-, and/or 45-day PHIs. HED concludes that the petitioner has provided adequate residue data. The results of the 1997 peanut field trials, wherein samples were analyzed within 30 days of harvest to minimize residue degradation, support the proposed tolerances of 1.0 ppm in/on peanut nutmeat and 0.6 ppm in/on peanut hay. However, the correct commodity definition are "Peanut" and "Peanut, hay." The Agency has also determined that "calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate" is the preferred chemical name for prohexadione-calcium (Memo 5/18/99, H. Podall; D253852). **A revised Section F is required.**

### Pome fruits

BASF submitted one volume of apple field trial data and one volume of pear field trial data (citations listed below) to support the establishment of the proposed crop group tolerance for residues of prohexadione-calcium in/on pome fruits.

44725220 Wofford, J.; Abdel-Baky, S.; Hess, E. et al. (1998) Magnitude of Prohexadione-Calcium Residues in Apples: Amended Report: Lab Project Number: 98/5202: 96011: 96058. Unpublished study prepared by BASF Corporation.

44725221 Wofford, J.; Hess, E.; Riley, M. (1998) Magnitude of Prohexadione-Calcium Residues in Pears: Lab Project Number: 98100: 98/5205: D9810. Unpublished study prepared by BASF Corporation.

Twenty apple field trials and eight pear field trials were conducted. Apple field trials were conducted during the 1996 growing season using nine different varieties in CA(2), CO(1), ID(2), NC(1), NY(2), OR(2), PA(2), MI(2), UT(1), VA(1), WA(2), and WI(2). Pear field trials were conducted during the 1998 growing season using four different varieties in CA(3), ID(1), NY(1), OR(2), and WA(1).

At each **apple** site, two separate plots of established apple trees received two broadcast foliar applications, with a 21-day retreatment interval, of the 27.5% DF formulation with adjuvant (Latron B1956 and ammonium sulfate) at either 0.85 lb ai/A/application (1.7 lb ai/A/season; 1x the maximum proposed seasonal application rate) or 1.7 lb ai/A/application (3.4 lb ai/A/season; 2x). Applications were made in spray volumes of ~50 gal/A (concentrated) or ~250 gal/A (dilute) using ground equipment (commercial or small plot equipment). An additional plot at each site was left untreated and served as a control.

At each **pear** site, one plot of established pear trees received a single broadcast foliar application of the 27.5% DF formulation with adjuvant (Latron B1956 and ammonium sulfate) at 1.7 lb ai/A (1x). Applications were made in spray volumes of ~250-350 gal/A (dilute) using ground equipment (commercial or small plot equipment). An additional plot at each site was left untreated and served as a control.

Whole mature apple and pear fruit samples were collected from each treatment plot 44-46 days following the last application. For residue decline studies, samples of apples were also harvested at 10-, 25-, and 55-day PTIs in the two field trials conducted in NY and WA, and samples of pears were also harvested at 24-, 35-, 55-, and 65-day PTIs in one field trial conducted in WA. The additional samples collected in two apple decline trials were within -35, -20, and 10 days of the proposed PHI (nominal harvest), and the additional samples collected in the pear decline trial were within -21, -10, 10, and 20 days of the proposed PHI (nominal harvest).

Single control and replicate treated samples were collected by hand from each test plot. Samples were placed in coolers with blue ice and frozen within 4 hours of harvest. All samples were shipped frozen to the analytical laboratory, BASF Agricultural Products Center (APC; Research Triangle Park, NC), where samples were stored frozen (<-10 C) until analysis. Total storage intervals for samples from the apple and pear field trials are presented in Table 21.

Samples of whole apple and pear fruit were homogenized at APC and stored frozen in plastic bags until analysis. Homogenized samples were analyzed for residues of prohexadione-calcium (quantitated as the prohexadione methyl ester) using Method D9608. The reported

LOQ for prohexadione-calcium was 0.05 ppm for apples and pears. Apparent residues of prohexadione-calcium were below the LOQ (<0.05 ppm) in/on 29 samples of untreated apple and 9 untreated samples of pear. Residues in/on treated apple and pear samples are presented in Tables 24 and 25, respectively.

Samples were also analyzed for the oxidative metabolite BX 112-I5, which was detected in the apple metabolism study. Samples were analyzed for residues of the metabolite BX 112-I5 using the HPLC/UV method (Method D9810). The reported LOQ for BX 112-I5 was 0.05 ppm for apples and pears. Residues of the metabolite BX 112-I5 were less than the LOQ (<0.05 ppm) in/on all untreated and treated samples of apple (n=21 controls and 52 treated samples) and pear (n=8 controls and 24 treated samples), and are therefore not reported in the tables below.

Table 24. Residues of prohexadione-calcium in/on **apples** following either two broadcast foliar applications of the 27.5% DF formulation at 0.85 lb ai/A/application (1x) or 1.7 lb ai/A/application (2x).

| County, State (EPA Region)<br>Study Number  | GPA <sup>a</sup> | PHI<br>(days) | Prohexadione (free<br>acid) Residues (ppm) | Prohexadione-Calcium<br>Residues (ppm) <sup>b</sup> |
|---|------------------|---------------|--|---|
| <b>Two applications at 0.85 lb ai/A/application (1x the maximum proposed seasonal application rate)</b> |                  |               |  |   |
| Tulare, CA (Region 10) 96033  | 49, 51           | 46            | 0.17, 0.17                                 | 0.201, 0.201  |
| Sutter, CA (Region 10) 96034  | 248, 249         | 45            | <0.05, <0.05                               | <0.05, <0.05  |
| Delta, CO (Region 9) 96031  | 50               | 45            | 0.12, 0.21                                 | 0.142, 0.248  |
| Payette, ID (Region 11) 96037   | 50, 51           | 45            | 0.52, 0.57                                 | 0.614, 0.673  |
| Payette, ID (Region 11) 96038   | 241, 248         | 45            | 0.48, 0.53                                 | 0.566, 0.625  |
| Kent, MI (Region 5) 96027   | 50               | 45            | 0.80, 1.06                                 | 0.944, 1.251  |
| Ottawa, MI (Region 5) 96028   | 249, 250         | 45            | 0.27, 0.28                                 | 0.319, 0.330  |
| Stokes, NC (Region 2) 96025   | 50               | 45            | 0.51, 0.65                                 | 0.602, 0.767  |
| Wayne, NY (Region 1) 96021  | 50, 51           | 10            | 0.82, 0.91                                 | 0.968, 1.074  |
|   |                  | 25            | 0.65, 0.83                                 | 0.767, 0.979  |
|   |                  | 45            | 0.50, 0.52                                 | 0.590, 0.614  |
|   |                  | 55            | 0.44, 0.76                                 | 0.519, 0.897  |
| Wayne, NY (Region 1) 96022  | 250, 251         | 45            | 0.76, 0.91                                 | 0.897, 1.074  |
| Hood River, OR (Region 11)<br>96039   | 50               | 45            | 1.92, 2.23 <sup>c</sup>                    | 2.266, 2.631  |
| Wasco, OR (Region 11) 96040   | 251, 253         | 45            | 0.11, 0.12                                 | 0.130, 0.142  |
| Lehigh, PA (Region 1) 96023   | 50, 51           | 45            | 0.63, 1.16                                 | 0.743, 1.369  |
| Berks, PA (Region 1) 96024  | 248, 250         | 45            | 0.44, 0.51                                 | 0.519, 0.602  |
| Cache, UT (Region 9) 96032  | 247, 252         | 45            | 0.17, 0.25                                 | 0.201, 0.295  |
| Carroll, VA (Region 2) 96026  | 248, 254         | 45            | 0.36, 0.45                                 | 0.425, 0.531  |

Table 24 (continued).

| County, State (EPA Region)<br>Study Number   | GPA <sup>a</sup> | PHI<br>(days) | Prohexadione (free<br>acid) Residues (ppm) | Prohexadione-Calcium<br>Residues (ppm) <sup>b</sup> |
|--|------------------|---------------|--|---|
| Grant, WA (Region 11) 96035  | 50, 51           | 10            | 0.70, 0.77                                 | 0.826, 0.909  |
|  |                  | 25            | 0.42, 0.65                                 | 0.496, 0.767  |
|  |                  | 45            | 0.37, 0.73                                 | 0.437, 0.861  |
|  |                  | 55            | 0.43, 0.59                                 | 0.507, 0.696  |
| Grant, WA (Region 11) 96036  | 250              | 45            | 0.10, 0.73                                 | 0.118, 0.861  |
| Dun, WI (Region 5) 96029   | 51               | 45            | 0.52, 0.53                                 | 0.614, 0.625  |
| Pierce, WI (Region 5) 96030  | 242, 255         | 45            | 0.09, 0.10                                 | 0.106, 0.118  |
| <b>Two applications at 1.7 lb ai/A/application (2x the maximum proposed seasonal application rate)</b> |                  |               |  |   |
| Tulare, CA (Region 10) 96033   | 50               | 46            | 0.52, 0.54                                 | 0.614, 0.637  |
| Sutter, CA (Region 10) 96034   | 251              | 45            | <0.05, 0.13                                | <0.05, 0.153  |
| Delta, CO (Region 9) 96031   | 50               | 45            | 0.27, 0.60                                 | 0.319, 0.708  |
| Payette, ID (Region 11) 96037  | 50               | 45            | 0.60, 1.21                                 | 0.708, 1.428  |
| Payette, ID (Region 11) 96038  | 249, 251         | 45            | 1.21, 1.77                                 | 1.428, 2.089  |
| Kent, MI (Region 5) 96027  | 50               | 45            | 2.46, 2.90 <sup>c</sup>                    | 2.903, 3.422  |
| Ottawa, MI (Region 5) 96028  | 250              | 45            | 1.28, 1.56                                 | 1.510, 1.841  |
| Stokes, NC (Region 2) 96025  | 50               | 45            | 0.91, 0.92                                 | 1.074, 1.086  |
| Wayne, NY (Region 1) 96021   | 50, 51           | 10            | 2.56, 2.77 <sup>c</sup>                    | 3.021, 3.269  |
|  |                  | 25            | 1.38, 1.50                                 | 1.628, 1.770  |
|  |                  | 45            | 1.29, 1.42                                 | 1.522, 1.676  |
|  |                  | 55            | 1.55, 1.57                                 | 1.829, 1.853  |
| Wayne, NY (Region 1) 96022   | 250, 251         | 45            | 0.21 <sup>d</sup> , 1.62                   | 0.248, 1.912  |
| Hood River, OR (Region 11)<br>96039  | 50               | 45            | 4.29, 5.46 <sup>c</sup>                    | 5.062, 6.443  |
| Wasco, OR (Region 11) 96040  | 250, 253         | 45            | 0.58, 0.58                                 | 0.684, 0.684  |
| Lehigh, PA (Region 1) 96023  | 51               | 45            | 1.26, 1.59                                 | 1.487, 1.876  |
| Berks, PA (Region 1) 96024   | 250, 251         | 45            | 1.10, 1.29                                 | 1.298, 1.522  |
| Cache, UT (Region 9) 96032   | 246, 253         | 45            | 0.34, 0.57                                 | 0.401, 0.673  |
| Carroll, VA (Region 2) 96026   | 245, 251         | 45            | 1.21, 1.28                                 | 1.428, 1.510  |
| Grant, WA (Region 11) 96035  | 50               | 10            | 3.54, 3.72 <sup>c</sup>                    | 4.177, 4.390  |
|  |                  | 25            | 2.14, 3.15 <sup>c</sup>                    | 2.525, 3.717  |
|  |                  | 45            | 3.09, 3.20 <sup>c</sup>                    | 3.646, 3.776  |
|  |                  | 55            | 1.83, 3.03 <sup>c</sup>                    | 2.159, 3.575  |
| Grant, WA (Region 11) 96036  | 251              | 45            | 0.44, 0.56                                 | 0.519, 0.661  |
| Dun, WI (Region 5) 96029   | 50, 51           | 45            | 1.68, 1.95 <sup>c</sup>                    | 1.982, 2.301  |
| Pierce, WI (Region 5) 96030  | 253, 254         | 45            | 0.39, 0.41                                 | 0.460, 0.484  |

<sup>a</sup> Applications were made either using concentrated (~50 gal/A) or dilute (~250 gal/A) spray volumes.

<sup>b</sup> Prohexadione-calcium residues were calculated by the study reviewer using a MWCF of 1.18 (acid to calcium).

<sup>c</sup> The highest residue value of replicate injections is reported.

<sup>d</sup> The petitioner considered this residue value to be an outlier.

Table 25. Residues of prohexadione-calcium in/on **pears** following a single broadcast foliar application of the 27.5% DF formulation at 1.7 lb ai/A/application (1x the maximum seasonal application rate).

| County, State (EPA Region)<br>Study Number | GPA <sup>a</sup> | PHI<br>(days) | Prohexadione-Calcium<br>Residues (ppm)       |
|--|------------------|---------------|--|
| Sutter, CA (Region 10) 98264               | 277              | 44            | 0.53, 0.54 <sup>b</sup>                      |
| Tulare, CA (Region 10) 98265               | 294              | 45            | 0.39, 0.52                                   |
| Tulare, CA (Region 10) 98266               | 262              | 45            | 0.23 <sup>b</sup> , 0.26                     |
| Payette, ID (Region 11) 98267              | 248              | 45            | 0.25, 0.32                                   |
| Wayne, NY (Region 1) 98263                 | 349              | 45            | 0.70 <sup>b</sup> , <b>0.99 <sup>b</sup></b> |
| Hood River, OR (Region 11) 98269           | 277              | 45            | 0.47, 0.56                                   |
| Hood River, OR (Region 11) 98270           | 292              | 45            | 0.57, 0.73 <sup>b</sup>                      |
| Grant, WA (Region 11) 98268                | 249              | 24            | 2.08, 2.11                                   |
|  |                  | 35            | 1.22, 1.56                                   |
|  |                  | 45            | 0.26, 0.95                                   |
|  |                  | 55            | 0.57, 0.78                                   |
|  |                  | 65            | 0.44, 0.55                                   |

<sup>a</sup> Applications were made using dilute (~250-350 gal/A) spray volumes.

<sup>b</sup> The highest residue value of replicate injections is reported.

Geographic representation of residue data for pome fruits is adequate. The current guidance (OPPTS 860.1500, Tables 2 and 5) recommends that a minimum of 18 trials (12 trials for apple in Regions 1 (3 trials), 2 (1 trials), 5 (2 trials), 9 (1 trial), 10 (1 trial), and 11 (4 trials); and 6 trials for pear in Regions 1 (1 trial), 10 (2 trials), and 11 (3 trials)) should be conducted for the establishment of a crop group tolerance for pome fruits. Twenty field trials for apples were conducted in Regions 1 (4 trials), 2 (2 trials), 5 (4 trials), 9 (2 trials), 10 (2 trials), and 11 (6 trials). Eight field trials for pears were conducted in Regions 1 (1 trial), 10 (3 trials), and 11 (4 trials).

### Conclusions

Residues of prohexadione-calcium were <0.05-2.631 ppm in/on apples harvested 45-46 days (the proposed PHI) following the last of two broadcast foliar applications of the 27.5% DF formulation at 0.85 lb ai/A/application (1.7 lb ai/A/season; 1x). Residues of prohexadione-calcium were 0.23-0.99 ppm in/on pears harvested 44-45 days following a single broadcast foliar application of the 27.5% DF formulation at 1.7 lb ai/A (1x). The petitioner has provided adequate residue data reflecting the maximum proposed use pattern for prohexadione-calcium on pome fruits. **Pending submission of confirmatory storage stability data**, the residue data submitted for apples and pears support the establishment of the proposed crop group tolerance for residues of prohexadione-calcium in/on pome

fruits at 3.0 ppm. However, the correct commodity definition is "Fruit, pome, group." **A revised Section F is required.**

Residues of the oxidative metabolite BX 112-I5, which was detected in the apple metabolism study, were less than the LOQ (<0.05 ppm) in all treated samples of apple and pear harvested 45 days following treatment at 1x the maximum proposed seasonal application rate. The petitioner proposes that these data represent field conditions; therefore, the metabolite BX 112-I5 is not a residue of concern. RAB1 will defer to the HED MARC on the toxicological significance of the prohexadione-calcium metabolites.

Additional data were provided demonstrating residues of prohexadione-calcium in/on apples treated at 2x the maximum proposed seasonal application rate. Residues of prohexadione-calcium were <0.05-6.443 ppm in/on apples harvested 45-46 days following the last of two broadcast foliar applications of the 27.5% DF formulation at 1.7 lb ai/A/application (3.4 lb ai/A/season; 2x). These data suggest that residue levels are proportional to the application rate.

Residue decline data indicate that generally, residues of prohexadione-calcium decline over time. Residues were highest at the 10-day PHI and declined to the lowest levels at the 45-, and 55- (apples) or 65-day (pears) PHIs.

Residue levels were not affected by applications made with either concentrated (~50 gal/A) or diluted (~250-350 gal/A) spray volumes. Residues of prohexadione-calcium were 0.142-2.631 ppm and <0.05-1.074 ppm in/on apples harvested 45 days following treatment at 1x the maximum proposed seasonal application rate in concentrated and dilute spray volumes, respectively.

#### OPPTS GLN 860.1520: Processed Food/Feed

##### Apples

BASF Corporation submitted one volume of data depicting the potential for concentration of residues of prohexadione-calcium in the processed commodities of apples. The citation is listed below.

44725222 Wofford, J.; Abdel-Baky, S.; Hess, E. et al. (1998) Magnitude of Prohexadione-Calcium Residues in Apple Processed Fractions: Amended Report: Lab Project Number: 96012: 98/5201: B96012. Unpublished study prepared by BASF Corporation.

A single apple field trial was conducted during the 1996 growing season in WA in order to generate samples for the processing study. Three separate plots were treated at different exaggerated rates.

Mature apple fruits were harvested 45 days following the last of two sequential foliar applications of the 27.5% DF formulation at 1.7 lb ai/A/application (3.4 lb ai/A/season; 2x the maximum proposed seasonal application rate); 5.1 lb ai/A/application (10.2 lb ai/A/season; 6x); or 8.5 lb ai/A/application (17.0 lb ai/A/season; 10x). The test substance was tank mixed with spray adjuvants (Latron B-1956 and ammonium sulfate) and applied in ~250 gal/A using a commercial ground boom sprayer with a 21-day retreatment interval. Since no phytotoxicity was observed with any of the exaggerated rates, only the 10x treatment was harvested for processing. A separate plot was not treated and served as a control.

Replicate untreated and 10x-treated samples of apples were harvested by hand. Whole apple samples were bagged and placed into coolers with ice. Samples were hand delivered to the processing facility (Wm. J. Englar and Associates, Inc., Moses Lake, WA) and frozen within 4 hours of harvest. Apple samples were processed on the day of receipt according to simulated industrial procedures. Briefly, whole apples were initially washed with cold water for five minutes. The washed apples were then reduced to crushed apple pulp using a hammermill assembly. The crushed apple pulp was heated with low steam to 40-50 C, and enzyme was added. After approximately 2 hours, the enzyme-treated apple pulp was pressed using a fruit press, and wet pomace and juice were collected. The juice was filtered to remove coarse solids. The moisture content of the collected wet pomace was determined to be within the specifications (60% moisture), and tray air drying was not necessary. The petitioner submitted adequate descriptions and material balance sheets for the processing procedures.

At the processing facility, the collected samples of apples (unwashed and washed), wet pomace, and juice were frozen and shipped within 3 days by overnight delivery (FedEx) to BASF Agricultural Products Center (APC, Research Triangle Park, NC). At BASF samples were frozen (<-10 C) until being homogenized; once homogenized for consistency, samples were frozen until residue analysis. Total storage intervals for samples from the apple processing study are presented in Table 21.

Samples of apples and the processed commodities (wet pomace and juice) were analyzed for residues of prohexadione-calcium (quantitated as the prohexadione methyl ester) using Method D9608. The reported LOQ was 0.05 ppm for all apple matrices. Apparent residues of prohexadione-calcium were less than the LOQ (<0.05 ppm) in/on one sample each of untreated apple and wet pomace and juice processed from untreated apple. Residues of prohexadione (free acid) in/on treated samples are presented in Table 26.

Samples of apple were also analyzed for the oxidative metabolite BX 112-I5 of prohexadione, which was detected in the apple metabolism



study. Samples were analyzed for residues of the metabolite BX 112-I5 using HPLC/UV method (Method D9810). The reported LOQ for BX 112-I5 was 0.05 ppm for all apple matrices. Residues of the metabolite BX 112-I5 were less than the LOQ (<0.05 ppm) in all untreated and treated samples of whole unwashed apples. Because detectable residues were not observed in the RAC treated at the exaggerated rate (10x), the processed apple fractions were not analyzed for residues of BX 112-I5.

Table 26. Residues of prohexadione (free acid) in the processed commodities of **apple** harvested 45 days following two foliar applications of the 27.5% DF formulation at ~8.5 lb ai/A/application (10x).

| Substrate        | Prohexadione (free acid) Residues (ppm) <sup>a</sup> | Concentration/Reduction Factor <sup>b</sup> |
|------------------|--|---|
| Apple (unwashed) | 8.52, 8.65 (8.59)                                    | --  |
| Apple (washed)   | 6.53, 7.09 (6.81)                                    | --  |
| -Wet pomace      | 6.49, 8.98 (7.74)                                    | 0.9x  |
| -Juice           | 4.73, 5.27 (5.00)                                    | 0.6x  |

<sup>a</sup> Averages are reported in parentheses.

<sup>b</sup> Concentration/reduction factors were calculated using the average values.

## Conclusions

The apple processing data are adequate for the purposes of this petition. No concentration of prohexadione-calcium residues was observed in wet pomace and juice processed from apples bearing detectable residues. Based on the results of the current processing study, tolerances for residues of prohexadione-calcium in the processed commodities of apples are not required.

Residues of the oxidative metabolite BX 112-I5 of prohexadione, which was detected in the apple metabolism study, were less than the LOQ (<0.05 ppm) in all untreated and treated samples of whole unwashed apples. Because detectable residues were not observed in the RAC treated at the exaggerated rate (10x), the processed apple fractions were not analyzed for residues of BX 112-I5. Based on these data, the petitioner concludes that BX 112-I5 is not a metabolite of concern in apples.

## Peanuts

BASF Corporation submitted one volume of data depicting the potential for concentration of residues of prohexadione-calcium in the processed commodities of peanuts. The citation is listed below.

44457810 Wofford, J.; Abdel-Baky, S.; Riley, M. (1996)  
Magnitude of Prohexadione Residues in Peanut Process

Fractions: Lab Project Number: 94158: 96/5207: ER96019.  
Unpublished study prepared by BASF Corp.

Two peanut field trials were conducted during the 1995 growing season in GA and NC in order to generate samples for the processing study. At each site, three separate plots were treated at different rates. Mature whole peanuts were harvested 25 days following the last of three sequential foliar applications of the 75% DF formulation at 0.125 lb ai/A/application (0.375 lb ai/A/season; 1x the maximum proposed seasonal application rate); 0.375 lb ai/A/application (1.125 lb ai/A/season; 3x); or 0.625 lb ai/A/application (1.875 lb ai/A/season; 5x). The test substances were tank mixed with spray adjuvants (crop oil concentrate and UAN) and applied in ~250 gal/A using a commercial ground boom sprayer with 21-day retreatment intervals. Since no phytotoxicity was observed with any of the exaggerated rates, only the 5x treatment was harvested for processing. A separate plot at each site was not treated and served as a control.

Peanut plants were dug by hand and inverted for five to seven days of field drying (PHI includes field drying time). Peanuts were mechanically collected using a combine and spread on a tarp. Replicate untreated and 5x-treated samples were randomly collected, bagged, and frozen. Samples were delivered within 2-11 days of harvest, on dry ice via FedEx to the processing facility (Food Protein Research and Development Center, Texas A&M University, Bryan, TX). The samples were stored frozen approximately 6.5 months prior to processing. Peanut samples were processed according to simulated industrial procedures. Briefly, whole unshelled peanuts were oven dried at 54-71 C until the moisture content was 7-12%. Light impurities were removed by aspiration, and small to large particles were removed by screening. The whole peanut was fed through a sheller and aspirator to separate the hull from the nutmeat. The nutmeat was oven dried at 54-71 C until the moisture content was 7-10%. A subsample of dried nutmeat was moisture conditioned to 12 %, heated to 93-104 C, and pressed in an expeller to extract crude oil. Following pressing, the presscake was flaked, and then extracted with hexane at 49-60 C for 30 minutes. The remaining presscake was washed twice again for 15 minutes with fresh hexane. Warm air was forced through the extracted presscake (meal) to remove residual hexane. The previously collected crude oil and hexane extracts were passed through a recovery unit to separate the crude oil from the hexane. The recovered crude oil was heated to 73-90 C to remove residual hexane, and refined. Refined oil and soapstock were then separated. The petitioner submitted adequate descriptions and material balance sheets for the processing procedures.

At the processing facility, the collected RAC, dried nutmeat, meal, and refined oil samples were frozen and shipped on the day processing was completed by overnight delivery (FedEx) to BASF

Agricultural Products Center (APC, Research Triangle Park, NC). At APC, samples were frozen (<-20 C) until being homogenized; once homogenized for consistency, samples were frozen until residue analysis. Total storage intervals for samples from the peanut processing study are presented in Table 21.

Only samples of peanuts and the processed commodities from the 5x treatment plot from GA were analyzed for residues of prohexadione-calcium (quantitated as the prohexadione methyl ester) using Method D9601. The reported LOQ was 0.05 ppm for all peanut matrices. Apparent residues of prohexadione-calcium were less than the LOQ (<0.05 ppm) in/on one sample each of untreated peanut and dried nutmeat, and meal and refined oil processed from untreated peanuts. Residues of prohexadione (parent acid) in/on treated samples are presented in Table 27.

Table 27. Residues of prohexadione (free acid) in the processed commodities of **peanuts** harvested 25 days following three foliar applications of the 75% DF formulation at ~0.625 lb ai/A/application (5x).

| Substrate                    | Prohexadione (free acid) Residues (ppm) <sup>a</sup> | Concentration/Reduction Factor <sup>b</sup> |
|------------------------------|--|---|
| Peanuts                      | 0.790, 0.876 (0.83)                                  | --  |
| -Nutmeat, dried <sup>c</sup> | 0.503, 0.586 (0.54)                                  | 0.7x  |
| -Meal                        | 0.403, 0.504 (0.45)                                  | 0.5x  |
| -Refined oil                 | <0.05, <0.05 (<0.05)                                 | <0.1x                                       |

<sup>a</sup> Averages are reported in parentheses.

<sup>b</sup> Concentration/reduction factors were calculated by using the average values.

<sup>c</sup> Dried nutmeat is not a RAC of peanuts according to the Table 1 of OPPTS 860.1000.

## Conclusions

The peanut processing data are adequate for the purposes of this petition. No concentration of prohexadione-calcium residues was observed in meal and refined oil processed from peanuts bearing detectable residues. Based on the results of the current processing study, tolerances for residues of prohexadione-calcium in the processed commodities of peanuts are not required.

## OPPTS GLN 860.1480: Meat, Milk, Poultry, Eggs

### Dairy Cattle Feeding Study

BASF submitted a study (citation listed below) depicting the magnitude of prohexadione-calcium residues in the milk and tissues of dairy cattle. The in-life phase of the study was conducted by Southwest Bio-Labs, Inc. (Las Cruces, NM), and the analytical phase

was performed by BASF Agricultural Products Center (APC; Research Triangle Park, NC).

44457809 Wofford, J.; Abdel-Baky, S.; Riley, M. (1997) A Meat and Milk Magnitude of the Residue Study with BAS 125 W (Prohexadione Calcium) in Lactating Dairy Cows: Lab Project Number: 96096: 97/5080: 96169B. Unpublished study prepared by BASF Corp.

Holstein dairy cows were orally dosed once daily via balling gun for 29 consecutive days with gelatin capsules containing prohexadione-calcium at target levels equivalent to 8 ppm (Treatment 1 or low dose), 24 ppm (Treatment 2 or mid-level dose), or 80 ppm (Treatment 3 or high dose). The daily dose was prepared and calculated based on recorded individual average feed consumption (dry weight basis) of test animals one week prior to actual dosing. The dosing levels were confirmed by analyzing two extra capsules from each treatment group on a weekly basis for the duration of the study. The maximum theoretical dietary burdens of prohexadione-calcium to dairy and beef cattle are 2.0 and 3.4 ppm, respectively; refer to Table 28 for calculation of dietary burdens. The dietary burdens were calculated using livestock feed items associated with the proposed uses on apples and peanuts. The feeding levels of 8, 24, and 80 ppm are, therefore, equivalent to 4x, 12x, and 40x, respectively, the anticipated maximum dietary burden for dairy cattle and ~2x, ~7x, and ~24x the anticipated maximum dietary burden for beef cattle.

Table 28. Estimation (based on U.S. feeding practices as reflected in Table 1 of OPPTS 860.1000) of the maximum theoretical dietary burden of prohexadione-calcium to beef and dairy cattle.

| Feed Commodity    | Estimated Tolerance, ppm | % Dry Matter | Beef Cattle |             | Dairy Cattle |             |
|-------------------|--------------------------|--------------|-------------|-------------|--------------|-------------|
|                   |                          |              | % of Diet   | Burden, ppm | % of Diet    | Burden, ppm |
| Apple, wet pomace | 3.0 <sup>a</sup>         | 40           | 40          | 3.0         | 20           | 1.5         |
| Peanut, meal      | 1.0 <sup>a</sup>         | 85           | 15          | 0.12        | 15           | 0.12        |
| Peanut, hay       | 0.6 <sup>b</sup>         | 85           | 25          | 0.12        | 50           | 0.35        |
| TOTAL             |                          |              | 80          | 3.4         | 85           | 2.0         |

<sup>a</sup> The proposed tolerance for the RAC is used. The submitted processing studies indicate that residues of prohexadione-calcium did not concentrate in wet pomace processed from apples bearing detectable residues, and in peanut meal processed from peanuts bearing detectable residues.

<sup>b</sup> The proposed tolerance for peanut hay is used.

A total of 14 dairy cows were used in the study: three cows for Treatment 1, three cows for Treatment 2, five cows for Treatment 3, and three cows for control. The control animals received placebo gelatin capsules containing cellulose powder. The cows were milked twice daily (a.m. and p.m.) and received the treatment capsule after the morning milking. The cows were supplied with a feed

concentrate, and water was provided *ad libitum*. The petitioner submitted adequate information pertaining to daily food consumption, milk production, and general health of the test animals.

The afternoon and following morning milk collections were weighed individually and composited (in proportion to production) for each animal of the dose group. Milk was collected on Days -1, 1, 2, 4, 7, 10, 14, 17, 21, 24, 28, 30, 31, 32, and 33. Subsamples of milk collected on Day 28 from the high-dose cows were separated into cream and skim milk. Milk samples were frozen immediately following collection and shipped frozen weekly to the analytical laboratory. Animals were sacrificed within 4.5 hours of the final dose, except for two animals from the highest dose group which were maintained on a "no-treatment diet" after the 29th day of dosing and were sacrificed after 2 and 5 days of withdrawal. Samples of liver (distal portions of each lobe), kidneys, fat (mesenteric and peripheral), and muscle (thigh and loin) were collected after sacrifice, immediately frozen ( $<-15^{\circ}\text{C}$ ), and shipped frozen via FedEx or freezer truck to the analytical laboratory. Samples were stored frozen ( $<-10^{\circ}\text{C}$ ) until analysis. Analyses were conducted approximately within one month of sampling. No supporting storage stability data are required to support the storage intervals and conditions of milk and tissue samples from the ruminant feeding study.

The collected samples were analyzed for residues of prohexadione-calcium using the previously described Method D9608. Liver and kidney samples were also analyzed for residues of the despropionyl prohexadione metabolite (designated in this study as metabolite BW 125-5376), which was identified in the goat metabolism study. These methods are described under "Residue Analytical Methods" section of this document. They have been adequately validated and are suitable for collecting residue data on levels of prohexadione-calcium and/or despropionyl prohexadione in milk and cattle tissues. The reported LOQs were 0.01 and 0.05 ppm for prohexadione-calcium in milk and tissues, respectively; and 0.05 ppm for despropionyl prohexadione in liver and kidney.

Residues of prohexadione-calcium were below the LOQ ( $<0.01$  ppm) in each milk sample from Treatment 2 (mid-level dose), therefore milk samples from the low-level dose were not analyzed. Residues of prohexadione-calcium were below the LOQ ( $<0.01$  ppm) in each milk sample from Treatment 3 (high-level dose), except for one sample from Day 10 (0.011 ppm); residues of prohexadione-calcium were also below the LOQ ( $<0.01$  ppm) in skim milk and cream from Treatment 3 (high-level dose). Residues of prohexadione-calcium in whole milk, skim milk, and cream samples from Treatment 3 (high dose) as well as in tissues from all dosing levels are presented in Table 29. Residue values reported for milk and tissues are not corrected or adjusted for method recoveries. Apparent residues of prohexadione-

calcium were each less than the respective LOQ (<0.01 ppm for milk and <0.05 ppm for tissues) in untreated milk (n=21 samples), kidney (n=5), liver (n=4), fat (n=4), muscle (n=4), skim milk (n=1), and cream (n=1).

Residues of despropionyl prohexadione were not detected (<0.05 ppm) in liver, but were detected in kidney samples from Treatment 2 and 3 (mid- and high-level doses); these residues are also presented in Table 29. Apparent residues of despropionyl prohexadione were each less than the LOQ (<0.05 ppm) in samples of untreated kidney (n=5) and liver (n=3). Apparent residues of the same metabolite were detected in one sample of untreated liver (0.073 ppm). The petitioner considers this sample to be contaminated or misnumbered, and did not use this value for the control group.

Table 29. Residues of prohexadione-calcium and despropionyl prohexadione in dairy cattle matrices following oral administration of prohexadione-calcium at target feeding levels of 8 ppm, 24 ppm, and 80 ppm for 29 consecutive days.

| Target Dose Level (ppm) | Dosing or Sampling Day | Uncorrected Residues (ppm) <sup>a</sup> |   |
|-------------------------|------------------------|---|---|
|                         |                        | Prohexadione-calcium                    | Despropionyl prohexadione (BW 125-5376) |
| Milk                    |                        |   |   |
| 80                      | 1                      | <0.01 (5)                               | Not analyzed (N/A)                      |
|                         | 2                      | <0.01 (5)                               | N/A                                     |
|                         | 4                      | <0.01(5)                                | N/A                                     |
|                         | 7                      | <0.01 (5)                               | N/A                                     |
|                         | 10                     | <0.01 (4), 0.013 <sup>b</sup>           | N/A                                     |
|                         | 14                     | <0.01 (5)                               | N/A                                     |
|                         | 17                     | <0.01 (5)                               | N/A                                     |
|                         | 21                     | <0.01 (5)                               | N/A                                     |
|                         | 24                     | <0.01 (5)                               | N/A                                     |
|                         | 28                     | <0.01 (5)                               | N/A                                     |
|                         | 30                     | <0.01 (2)                               | N/A                                     |
|                         | 31                     | <0.01                                   | N/A                                     |
|                         | 32                     | <0.01                                   | N/A                                     |
|                         | 33                     | <0.01                                   | N/A                                     |
| Cream                   |                        |   |   |
| 80                      | 28                     | <0.01 (4)                               | N/A                                     |
| Skim Milk               |                        |   |   |
| 80                      | 28                     | <0.01 (4)                               | N/A                                     |
| Fat                     |                        |   |   |
| 8                       | 29                     | <0.05 (3)                               | N/A                                     |
| 24                      | 29                     | <0.05 (3)                               | N/A                                     |

Table 29 (continued).

| Target Dose Level (ppm) | Dosing or Sampling Day | Uncorrected Residues (ppm) <sup>a</sup>     |   |
|-------------------------|------------------------|---|---|
|                         |                        | Prohexadione-calcium                        | Despropionyl prohexadione (BW 125-5376)     |
| 80                      | 29                     | <0.05, 0.056, 0.079                         | N/A   |
|                         | 31                     | <0.05                                       | N/A   |
|                         | 34                     | <0.05                                       | N/A   |
| <b>Kidney</b>           |                        |   |   |
| 8                       | 29                     | 0.09, 0.097, 0.312                          | <0.05, <0.05, <0.05                         |
| 24                      | 29                     | 0.431, 0.638, 0.837                         | 0.080, 0.087, 0.094                         |
| 80                      | 29                     | 1.66, 2.74 <sup>b</sup> , 4.65 <sup>b</sup> | 1.78, 4.96 <sup>b</sup> , 5.14 <sup>b</sup> |
|                         | 31                     | <0.05                                       | <0.05                                       |
|                         | 34                     | <0.05                                       | <0.05                                       |
| <b>Liver</b>            |                        |   |   |
| 8                       | 29                     | <0.05 (3)                                   | <0.05 (3)                                   |
| 24                      | 29                     | <0.05, 0.05, 0.051                          | <0.05, <0.05, <0.05                         |
| 80                      | 29                     | <0.05, 0.077, 0.208                         | <0.05, <0.05, <0.05                         |
|                         | 31                     | <0.05                                       | <0.05                                       |
|                         | 34                     | <0.05                                       | <0.05                                       |
| <b>Muscle</b>           |                        |   |   |
| 8                       | 29                     | <0.05 (3)                                   | N/A   |
| 24                      | 29                     | <0.05 (3)                                   | N/A   |
| 80                      | 29                     | <0.05 (2), 0.081                            | N/A   |
|                         | 31                     | <0.05                                       | N/A   |
|                         | 34                     | <0.05                                       | N/A   |

<sup>a</sup> Each residue value represents a single sample (individual cow) unless otherwise noted in parentheses; residue values for prohexadione-calcium and despropionyl prohexadione are listed respectively.

<sup>b</sup> The highest value of replicate injections is reported.

### Conclusions

The cattle feeding study is acceptable. Dairy cows were orally dosed once daily with prohexadione-calcium at 8, 24, or 80 ppm (4x, 12x, and 40x, respectively, the anticipated maximum dietary burden of 2.0 ppm for dairy cattle and ~2x, ~7x, and ~24x, respectively, the anticipated maximum dietary burden for beef cattle) for 29 consecutive days. The results of the study suggest that residues of prohexadione-calcium are not likely to transfer to milk (including cream and skim milk), fat, liver, and muscle when the chemical is used according to the proposed use directions. However, residues of prohexadione-calcium are expected to transfer to liver and kidney. By extrapolation of the average residues at

~2x, ~7x, and ~24x the anticipated maximum dietary burden for beef cattle, HED concludes that the proposed tolerance levels are 0.1 ppm for residues of prohexadione-calcium in cattle kidney and 0.05 ppm for meat byproducts (except kidney). The petitioner should submit a revised Section F to amend the proposed tolerance from "cattle, meat byproduct (kidney)" to "cattle, goats, hogs, horses, and sheep, kidney" and to add "cattle, goats, hogs, horses, and sheep, meat byproducts, except kidney."

Cow's kidney and liver samples were additionally analyzed for residues of despropionyl prohexadione, a metabolite identified in the kidney and liver of goats from the ruminant metabolism study. At the lowest feeding level, residues of despropionyl prohexadione in cow's kidney and liver were each nondetectable (<0.05 ppm). These data suggest that, at the current dietary burden, residues of the despropionyl metabolite are not likely to transfer to cow's kidney and liver when the chemical is used according to the proposed use directions.

#### Poultry Feeding Study

A poultry feeding study was not submitted with the subject petition. The only poultry feed item associated with this petition is peanut meal. The maximum theoretical dietary burden of prohexadione-calcium for poultry animals is calculated below in Table 30.

Table 30. Estimation (based on U.S. feeding practices as reflected in Table 1 of OPPTS 860.1000) of the maximum theoretical dietary burden of prohexadione-calcium for poultry animals.

| Feed Item    | Estimated Maximum Residue Level (ppm) | % of Diet | Estimated Contribution in Diet (ppm) |
|--------------|---------------------------------------|-----------|--------------------------------------|
| Peanut, meal | 1.0 <sup>a</sup>                      | 25        | 0.25                                 |
| Total        |                                       | 25        | 0.25                                 |

<sup>a</sup> The proposed tolerance for the RAC is used. The submitted peanut processing study indicates that residues of prohexadione-calcium did not concentrate in meal processed from peanuts bearing detectable residues.

The poultry metabolism study reviewed in this petition was conducted at feeding levels of 8.43 and 33.4 ppm which are respectively equivalent to 34x and 134x the maximum theoretical dietary burden for poultry. At the low-dose level of 8.43 ppm, the TRR in eggs and tissues were <0.01 ppm. At the high-dose level of 33.4 ppm, prohexadione was identified in egg yolks (12.5% TRR, 0.003 ppm), egg whites (27.3% TRR, 0.004 ppm), kidney (13.2% TRR, 0.062 ppm), and liver (14.9% TRR, 0.005 ppm). Assuming a linear relationship between dose and residues, the expected prohexadione-calcium residues in eggs and poultry tissues would be below the LOD for the methods used to measure residues in livestock products. Therefore, HED tentatively concludes that there is no reasonable



expectation of finite residues [§180.6(a)(3)] in eggs and poultry tissues. The HED MARC will be informed of this tentative conclusion but may require a poultry feeding study depending on the outcome of its deliberation. The petitioner is further advised that should dietary burden increase due to the addition of prohexadione-calcium treated poultry feed commodities through new uses, HED may require the submission of a poultry feeding study and the establishment of tolerances to cover secondary residues in eggs and poultry tissues.

#### OPPTS GLN 860.1850: Confined Accumulation in Rotational Crops

BASF has submitted data from a study (citation listed below) investigating the metabolism of [ $^{14}\text{C}$ ]prohexadione-calcium in rotational crops. The in-life and analytical phases of the study were conducted by Battelle (Columbus, OH).

44457792 Steginsky, C.; Venkatesh, K.; Powell, J. et al. (1996) Confined Rotational Crop Study with (carbon 14)-BAS 9054 W (Prohexadione-Calcium): Lab (Prohexadione-Calcium): Lab Project Number: SC930243: M93150: M9510. Unpublished study prepared by Battelle and Agvise Labs., Inc.

The radioactive test substance was prepared by isotopic dilution of [ $^{14}\text{C}$ ]-prohexadione-calcium (specific activity 61.1  $\mu\text{Ci}/\text{mg}$ , radiochemical purity >98%) with [ $^{13}\text{C}$ ]prohexadione-calcium; [ $^{14}\text{C}$ ] and [ $^{13}\text{C}$ ]prohexadione-calcium were labeled at the C-3 and C-5 positions of the cyclohexenone ring. The test substance was diluted with water to yield a final specific activity of 30.9  $\mu\text{Ci}/\text{mg}$  and radiochemical purity of 97%. It was then applied to containers (plywood flats lined with plastic sheeting) of sandy loam soil (81% sand, 15% silt, and 4% clay, 0.9% organic matter, pH 7.1, cation exchange capacity 3.5 meq/100 g) as a single application at ~0.343 lb ai/A/application (0.9x the maximum proposed seasonal application rate for peanuts) using hand-held  $\text{N}_2$ -pressured sprayers. The containers were maintained in a greenhouse and consisted of a total of three control flats (one flat for each rotated crop) and four treated flats (one treated flat for each rotated crop and an additional treated flat for the fall planting of wheat). Lettuce (leaf - Romaine), turnip, and wheat were planted to the plots 31 days after the final application (DAT) to simulate crop failure and emergency replanting, and 122 DAT for crops rotated immediately following harvest. A 365-day plantback interval (PBI) was not conducted due to low concentrations of residues in the soil and RACs at the 122-day PBI. Control flats were sprayed with a solution blank of HCl and acetonitrile diluted with water; control flats were only planted at the 31-day PBI. Water, fertilizer, and maintenance insecticides were applied to the crops as necessary.

Adequate information concerning preparation of the test substance, greenhouse conditions, and plant maintenance was provided.

Samples of immature wheat forage were collected 52 days after planting. The remaining RACs were collected at maturity: 63-77 days after planting for lettuce and turnips, and 126 days after planting for wheat. Lettuce tops were cut from the roots and composited as one sample. Turnip roots were separated from the tops, the soil brushed from the roots and the taproots removed. Wheat forage samples were collected by cutting the entire plant half-inch above the soil. The heads of mature wheat were removed from stalks before cutting the stalks half-inch above the soil. The wheat heads were threshed to separate grain from the chaff; the chaff was combined with the straw samples. Sample storage conditions were not discussed.

#### Total radioactive residues (TRR)

Rotational crop commodities were homogenized with liquid nitrogen and triplicate aliquots were subjected to combustion/LSC for TRR determinations. The TRR in rotational crop commodities are presented in Table 31; the LOQ for TRR determinations was reported as ~0.001 ppm.

Table 31. Total radioactive residues in samples of rotational crop commodities grown in soil treated with [<sup>14</sup>C]prohexadione-calcium at a total application rate of ~0.343 lb ai/A (presumably ~1x the maximum proposed seasonal application rate for peanuts).

| Commodity               | TRR, ppm [ <sup>14</sup> C]prohexadione equivalents |         |
|-------------------------|---|---------|
|                         | 31-DAT <sup>a</sup>                                 | 122-DAT |
| Lettuce                 | 0.0010  | 0.0006  |
| Turnip tops             | 0.0042  | 0.0008  |
| Turnip roots            | 0.0027  | 0.0015  |
| Wheat forage (immature) | 0.0070  | 0.0045  |
| Mature wheat grain      | 0.0141  | 0.0086  |
| Mature wheat straw      | 0.0237  | 0.0188  |

<sup>a</sup> DAT = Days after treatment.

#### Extraction of residues

Homogenized samples of rotational crop commodities with total radioactivity greater than 0.01 ppm (i.e., 31-DAT wheat grain and straw, and 122-DAT wheat straw) were subjected to extraction and hydrolysis procedures. During the fractionation procedures, aliquots of extracts, hydrolysates, and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. The general extraction procedures are summarized below.

Initially, subsamples of 31-DAT wheat grain and straw homogenates were repeatedly (4x) extracted with ACN:sulfuric acid (9:1, v:v), and the extracts were combined. The nonextractable residues were extracted (2x) further with hexane. A second extraction procedure was used to yield higher levels of extracted residues. The second procedure involved hydrating the wheat grain and straw homogenates in water overnight prior to extraction. Following hydration, samples of 31-DAT wheat grain and straw, and 122-DAT wheat straw were extracted as discussed above for unhydrated samples.

The petitioner validated the extraction procedures using control wheat grain and straw fortified with [ $^{14}\text{C}$ ]prohexadione-calcium at 0.0134 and 0.0238 ppm, respectively. The fortification levels were representative of the total residues found in treated wheat grain and straw. The fortified samples were hydrated and extracted (3x) with ACN:sulfuric acid (9:1, v:v); the majority of the radioactivity was determined in the ACN extracts (109% TRR, 0.0146 ppm in grain and 96.4% TRR, 0.0229 ppm in straw). Nonextractable residues were extracted with petroleum ether:ether (87:13, v:v), and the ACN extract was partitioned with methylene chloride:ACN (3:1, v:v) as for the residue analytical method. LSC analysis determined that the majority of the radioactivity was partitioned into the organic phase (97.2-111% TRR); HPLC analysis confirmed the residues as prohexadione-calcium by co-chromatography with [ $^{14}\text{C}$ ]prohexadione-calcium standard. The petitioner states these results demonstrate that [ $^{14}\text{C}$ ]prohexadione-calcium has good extractability and stability throughout the ACN extraction, concentration, and partitioning procedures.

#### Hydrolysis of nonextractable residues

The nonextractable residues remaining following hexane extraction of the 31-DAT wheat grain and straw were hydrolyzed with carbohydrate-digesting enzymes. Prior to enzyme hydrolysis, nonextractable residues were rinsed with 50 mM acetate buffer (pH 5) and centrifuged to remove residual acid and solubilize water-soluble natural products such as oligosaccharides, oligopeptides, and soluble proteins. The supernatant was reserved for LSC analysis, and additional acetate buffer and a mixture of carbohydrate-digesting enzymes (cellulase, hemicellulase,  $\alpha$ -amylase, amyloglucosidase, and pectinase) were added to the pellet. The mixture was incubated for 24 hours at 40 C and then centrifuged. The remaining pellet was again subjected to enzyme hydrolysis, and the hydrolysates reserved for LSC analysis.

Further characterization and metabolite identification was not performed as the enzyme hydrolyses determined that the majority of the radioactivity was associated with carbohydrates (120% TRR, 0.017 ppm) in wheat grain, and either carbohydrate associated (27.9% TRR, 0.007 ppm) or nonextractable (37.2% TRR, 0.009 ppm)

residues in wheat straw. All extracts other than those characterized by enzyme hydrolysis, and nonextractable residues were less than 0.01 ppm and did not require additional characterization/identification.

The petitioner validated the effects of the enzyme hydrolysis procedure in the absence of enzymes using control wheat grain and straw samples. Samples were incubated overnight using buffer without enzymes. No detectable residues were released in the enzyme control samples thus verifying that solubilization of residues in treated samples was the result of enzyme activity, specific for carbohydrates, and not due to non-specific buffer solubilization.

The distribution and characterization of  $^{14}\text{C}$ -activity in the extracts and hydrolysates of rotational crop commodities (wheat grain and straw) is presented in Table 32.

Table CROC (continued).

Table 32. Distribution and characterization of radioactive residues in rotational crop commodities grown in soil treated with [<sup>14</sup>C]prohexadione-calcium at 0.343 lb ai/A (presumably ~1x).

| Fraction                                      | % TRR           | ppm    | Characterization/Identification   |
|---|-----------------|--------|---|
| <b>31-DAT Wheat grain (TRR = 0.0141 ppm)</b>  |                 |        |   |
| <b>Unhydrated subsample</b>                   |                 |        |   |
| ACN   | 3.91            | 0.0006 | Not further analyzed (N/A).   |
| Nonextractable                                | NR <sup>a</sup> | NR     | Extracted (2x) with hexane.   |
| Hexane  | 1.34            | 0.0002 | N/A.  |
| Nonextractable                                | 92.6            | 0.0131 | N/A.  |
| <b>Hydrated subsample</b>                     |                 |        |   |
| ACN   | 9.10            | 0.0013 | N/A.  |
| Nonextractable                                | NR              | NR     | Extracted (2x) with hexane.   |
| Hexane  | 1.25            | 0.0002 | N/A.  |
| Nonextractable                                | 103             | 0.0145 | Rinsed with acetate buffer (pH 5); subjected to enzymatic hydrolysis with carbohydrate digesting enzymes (2x).  |
| Buffer rinsate                                | 6.2             | 0.0009 | N/A.  |
| Enzyme hydrolysate                            | 114             | 0.0161 | N/A.  |
| Nonextractable                                | 10.4            | 0.0015 | N/A.  |
| <b>31-DAT Wheat straw (TRR = 0.0237 ppm)</b>  |                 |        |   |
| <b>Unhydrated subsample</b>                   |                 |        |   |
| ACN   | 18.8            | 0.0045 | N/A.  |
| Nonextractable                                | NR              | NR     | Extracted (2x) with hexane.   |
| Hexane  | 1.28            | 0.0003 | N/A.  |
| Nonextractable                                | 71.9            | 0.0170 | N/A.  |
| <b>Hydrated subsample</b>                     |                 |        |   |
| ACN   | 34.1            | 0.0081 | N/A.  |
| Nonextractable                                | NR              | NR     | Extracted (2x) with hexane.   |
| Hexane  | 1.01            | 0.0002 | N/A.  |
| Nonextractable                                | 58.6            | 0.0139 | Rinsed with acetate buffer (pH 5); subjected to enzymatic hydrolysis with carbohydrate digesting enzymes. (2x). |
| Buffer rinsate                                | 6.2             | 0.0015 | N/A.  |
| Enzyme hydrolysate                            | 21.7            | 0.0052 | N/A.  |
| Nonextractable                                | 37.2            | 0.0088 | N/A.  |
| <b>122-DAT Wheat straw (TRR = 0.0188 ppm)</b> |                 |        |   |
| <b>Hydrated subsample</b>                     |                 |        |   |
| ACN   | 43.3            | 0.0081 | N/A.  |
| Nonextractable                                | NR              | NR     | Extracted (2x) with hexane.   |
| Hexane  | 0.76            | 0.0001 | N/A.  |
| Nonextractable                                | 57.1            | 0.0107 | N/A.  |

<sup>a</sup> NR = Not Reported.

### Storage stability

Unhydrated samples of rotated 31-DAT wheat grain and straw were extracted within 70-71 days (~2 months) of harvest; hydrated samples were extracted within 113-204 days (~4-7 months) of harvest. Greater extractabilities of residues were observed for hydrated samples which were stored for longer intervals. The petitioner contends that since the majority of residues in both the unhydrated and hydrated samples were nonextractable and that most of the nonextractable residues in hydrated wheat grain were associated with carbohydrates, no supporting storage stability data should be required. Based on the supporting storage stability data submitted for plant metabolism studies, HED concurs that no additional storage stability data are required to validate the results of the confined rotational crop study.

### Proposed metabolic pathway

Based on the available data, the petitioner proposes that prohexadione-calcium is metabolized in rotational crops by plant enzymes and incorporated into natural products such as carbohydrates.

### Study summary

The submitted confined rotational crop study is adequate. [<sup>14</sup>C]prohexadione-calcium was applied to sandy loam soil at 0.343 lb ai/A (0.9x the maximum proposed seasonal application rate for peanuts). Representatives of small grains (wheat), leafy vegetables (lettuce), and root crops (turnips) were planted 31- and 122-days after treatments (DAT). Total radioactive residues were <0.01 ppm in/on all rotational crop commodities except in 31-DAT wheat grain (0.0141 ppm), 31-DAT wheat straw (0.0237 ppm), and 122-DAT wheat straw (0.0188 ppm).

Rotational crop commodities with total radioactivity greater than 0.01 ppm were subjected to extraction and hydrolysis procedures in order to characterize/identify residues. No residues of prohexadione-calcium or related metabolites were identified. Enzyme hydrolyses demonstrated that the majority of the radioactivity was associated with carbohydrates (120% TRR, 0.017 ppm) in wheat grain, and either carbohydrate associated (27.9% TRR, 0.007 ppm) or nonextractable (37.2% TRR, 0.009 ppm) residues in wheat straw. All extracts other than those characterized by enzyme hydrolysis, and nonextractable residues were ≤0.01 ppm and did not require additional characterization/identification.

Based on the results of this study, HED concludes that limited rotational field studies are not required and a 30-day plantback restrictions is appropriate for the purpose of this petition. A

revised Baseline™ label, which includes a 30-day plantback restriction for all rotational crops (except peanuts), should be submitted.

#### Codex Issues

There is neither a Codex proposal, nor Canadian or Mexican limits for residues of prohexadione-calcium in/on plant or animal commodities. Therefore, no compatibility issues exist with regard to the proposed U.S. tolerances discussed in this petition review. A copy of the IRLS sheet is attached to this memorandum.

#### List of Attachments

- I. International Residue Limit Status Sheet
- II. Figure 1: Chemical names and structures of prohexadione and its metabolites identified in plant and animal metabolism studies.

**ATTACHMENT I**

International Residue Limit Status Form



## INTERNATIONAL RESIDUE LIMIT STATUS

|  |                                      |  |                   |
|--|--------------------------------------|--|-------------------|
| Chemical Name:<br>calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate | Common Name:<br>Prohexadione-Calcium | <input checked="" type="checkbox"/> Proposed tolerance<br><input type="checkbox"/> Reevaluated tolerance<br><input type="checkbox"/> Other | Date:<br>011/5/99 |
|--|--------------------------------------|--|-------------------|

|   |  |
|---|--|
| Codex Status (Maximum Residue Limits)   | U. S. Tolerances   |
| <input checked="" type="checkbox"/> No Codex proposal step 6 or above<br><input type="checkbox"/> No Codex proposal step 6 or above for the crops requested | Petition Number: PP#8F4941<br>DP Barcode: D252257<br>Other Identifier: |
| Residue definition: N/A   | Reviewer/Branch: G.F. Kramer   |
|   | Residue definition: parent   |

| Crop (s) | MRL (mg/kg) | Crop(s)                         | Tolerance (ppm) |
|----------|-------------|---------------------------------|-----------------|
|          |             | Peanut Nutmeat                  | 1.0             |
|          |             | Peanut Hay                      | 0.6             |
|          |             | Pome fruit                      | 3.0             |
|          |             | Cattle, Meat Byproduct (Kidney) | 0.1             |
|          |             |                                 |                 |

|   |   |
|---|---|
| Limits for Canada   | Limits for Mexico   |
| <input checked="" type="checkbox"/> No Limits<br><input type="checkbox"/> No Limits for the crops requested | <input checked="" type="checkbox"/> No Limits<br><input type="checkbox"/> No Limits for the crops requested |
| Residue definition: N/A   | Residue definition: N/A   |

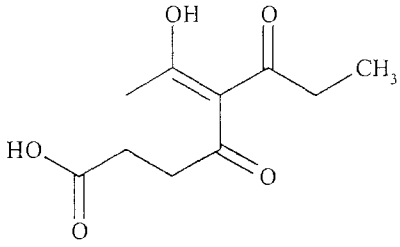
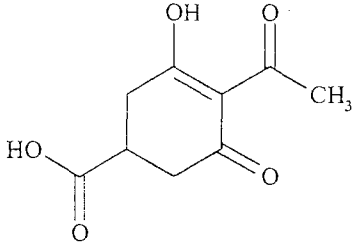
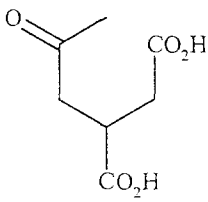
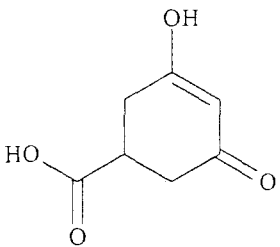
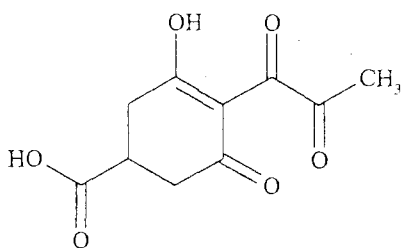
| Crop(s) | MRL (mg/kg) | Crop(s) | MRL (mg/kg) |
|---------|-------------|---------|-------------|
|         |             |         |             |
|         |             |         |             |

|                              |
|------------------------------|
| Notes/Special Instructions:. |
|------------------------------|

## ATTACHMENT II

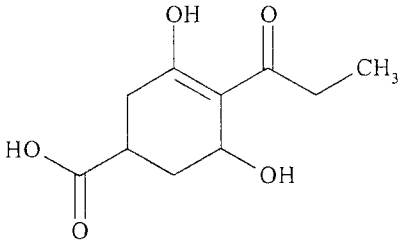
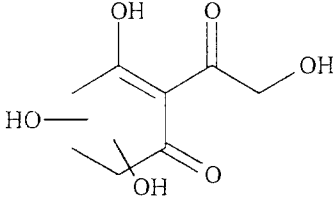
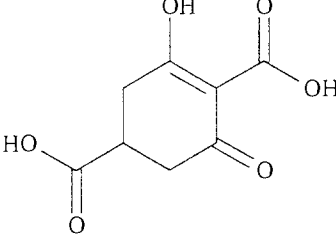
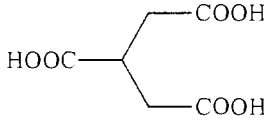
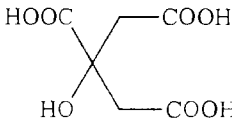
Figure 1: Chemical names and structures of prohexadione and its metabolites identified in plant and animal metabolism studies.

Figure 1. Chemical names and structures of prohexadione and its metabolites identified in plant and animal metabolism studies.

| Common Name or<br>Company Code<br>Chemical Name   | Structure  | Substrate   |
|---|--|---|
| Prohexadione (BAS 125 W,<br>free acid; BAS 9054 W) <sup>a</sup><br><br>3-oxido-5-oxo-4-<br>propionylcyclohex-3-<br>enecarboxylate |    | Apples<br><br>Peanut nutmeats, hulls,<br>and hay<br><br>Goat milk, kidney,<br>liver, muscle, and fat<br><br>Hen egg yolk and whites,<br>kidney, and liver |
| BX 112-I5<br>(BW125-31F)  |     | Apples  |
| BX 112-M10  |    | Apples  |
| Despropionyl<br>prohexadione<br>(referred to as BW 125-<br>5376 in the ruminant<br>feeding study)                                 |   | Apples<br><br>Peanut hulls and hay<br><br>Goat milk, kidney,<br>liver, muscle, and fat  |
| Dioxopropyl prohexadione  |  | Peanut nutmeats, hulls,<br>and hay  |

(continued)

Figure 1 (continued).

| Common Name or<br>Company Code<br>Chemical Name                        | Structure   | Substrate   |
|--|---|---|
| 27F2-B (and 45F2-A)<br>3,5-Dimethoxy-4-propionyl-methyl<br>benzoate    |   | Apples  |
| 25F1-A<br>2-Hydroxyacetyl-3-methoxy-dihydroxy-1-cyclohexenone          |    | Apples  |
| 27F2-A (and 27F1-A)<br>3,5-Dioxocyclohexane-1,4-dimethyl-dicarboxylate |   | Apples  |
| Tricarballic acid<br>(TCA)   |  | Apples<br>Peanut nutmeats, hulls,<br>and hay<br>Hen kidney and liver. |
| Citric acid  |  | Apples  |

BAS 125W was used interchangeably by the petitioner to refer to prohexadione-calcium or prohexadione (free acid).

